Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Two Lessons From the Interface of Genetics and Medicine

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Thoughts are but dreams till their effects be tried.
William Shakespeare, *The Rape of Lucrece*

While growing up in Kenya, I became interested in natural history, anthropology, and medicine. Natural history included Darwinism, and at Oxford University after World War II, I learned what was then a novel concept: that natural selection results from changes in gene frequencies in populations. The theoretical basis of population genetics and of the effects of selection had been provided by R. A. Fisher and J. B. S. Haldane in England and by Sewall Wright in the United States. My parallel interest was the diversity of indigenous peoples in East Africa, who belong to several linguistic and cultural groups. An attack of malaria forcibly directed my attention toward parasitic diseases and the need for doing something to relieve tropical maladies. The wish to participate in such a worthwhile task provided motivation for a career in medical research. These rather diverse interests coincided to produce my first major scientific contribution, which was published in 1954, 50 years ago.

THE DISCOVERY THAT SICKLE-CELL HETEROZYGOTES ARE RESISTANT TO MALARIA

James Herrick, a Chicago physician, observed sickle cells in the peripheral blood of an anemic dental student (Herrick 1910). The condition of the patient was termed "sickle-cell anemia," but because there are several other manifestations, the designation "sickle-cell disease" is now preferred. Studies by several investigators, reviewed elsewhere (Allison 2002a), established that sickling of red blood cells requires loss of oxygen and that the capacity to develop sickling is inherited as an autosomal dominant character. In patients with sickle-cell disease, sickling can occur in venous blood, whereas in the majority of carriers sickling is observed only when blood is strongly deoxygenated *in vitro*, for example, in the presence of a reducing agent.

The modern phase of research on sickle-cell disease began in 1949. Linus Pauling recalled how in 1945 he heard from W. B. Castle, a Harvard physician, about sickle cells and the need for deoxygenation to produce them. "It immediately occurred to me that sickle-cell anemia must be a disease of the hemoglobin molecule. . . . the molecules line up to form long thin strands . . . which would cause the cell to be deformed into the shape of a sickle or crescent" (Pauling 1994, p. xvii). This remarkable insight was confirmed when it was shown in his laboratory that hemoglobin (Hb) from patients with sickle-cell disease has a lower negative charge at physiological pH than does normal adult Hb (Pauling *et al.* 1949). The Hb's were designated S and A; the Hb from patients with sickle-cell disease was nearly all of the S type (apart from a minor component of fetal Hb). In parents and siblings there was a nearly equal amount of HbS and HbA, leading to the conclusion that they were AS heterozygotes, whereas the patients were SS homozygotes. This interpretation was confirmed by family studies published in the same year (Beet 1949; Neel 1949).

I entered the scene in mid-1949. By then I had completed basic science studies and had an interval of several months before starting clinical training in the medical school at Oxford. On an expedition from the university to Kenya, my role was to investigate blood groups and other inherited characteristics in East African tribes. One of the genetic markers studied was the sickle-cell trait. It was known that ~8% of African Americans carry this condition (Diggs *et al.* 1933), but little information was then available on the distribution of the trait in Africa. To my surprise, I found remarkably high frequencies of sickle-cell trait carriers (20–30%) in tribes living close to the coast of Kenya and near

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Lake Victoria, whereas in the intervening highlands the frequencies were <1%. These differences cut across linguistic and cultural boundaries and were independent of blood group frequencies, which we documented (Allison *et al.* 1952).

Such a distribution raised an interesting question. In populations with AS frequencies of 20–30%, SS frequencies of 1–>2% would be predicted. In keeping with this expectation, I was shown many cases of sickle-cell disease in pediatric wards of hospitals in Kisumu (near Lake Victoria) and Mombasa (on the Kenyan coast), in contrast to very few in Nairobi (central Kenya). Under rural African conditions, survival of SS homozygotes to reproductive age was exceptional, so that selection against this genotype must have been strong. For the lost genes to be replaced by mutation, the mutation rate would have to be unprecedented and confined to certain populations. Why, then, had the gene become common in some parts of Kenya but not others?

Faced with these facts at the end of the 1949 expedition, I had my own flash of inspiration. A common environmental factor in the regions near Lake Victoria and the coast is intense transmission of the malaria parasite *Plasmodium falciparum*, which in one phase of its life cycle multiplies within red blood cells. Sickle-cell heterozygotes might be relatively resistant to this type of malaria, so that their chances of surviving repeated attacks in early childhood would be increased. By this mechanism, the fitness of AS heterozygotes could be greater than that of AA homozygotes, resulting in a stable polymorphism. Testing this exciting hypothesis had to wait until I had completed my medical studies and received training in parasitology.

The opportunity eventually came in 1953, when I spent nearly a year in East Africa working on the project, which must be placed in context. The parasite *P. falciparum*, which produces the most severe forms of malaria, is transmitted by *Anopheles gambiae* and related mosquitoes. The vectors flourish in hot, humid environments such as the coastal regions of Kenya and Tanzania, the region around Lake Victoria, and low-altitude tropical forests. The vectors cannot survive in the highlands or arid regions of East Africa. African infants living in hyperendemic areas have few malaria attacks during the first months of life because they receive some shelter from mosquitoes and fetal Hb, and maternal antibodies may provide some protection. Children aged 4 months to 4 years suffer repeated attacks, with severe morbidity and appreciable mortality. Potentially lethal forms of malaria (usually cerebral malaria or severe anemia) nearly always occur in children with high parasitemia (Field 1949; Greenwood *et al.* 1991). From school age to adulthood, Africans living in hyperendemic areas have a high level of acquired immunity. Another problem in studying malaria epidemiology is the random use of over-the-counter antimalarial drugs.

In 1953 it was widely held that immunity to parasites resulted from "premunition," which was maintained by persistent infection or by reinfection. Consequently, immunity to malaria would rapidly decline in persons moving from a malarious environment to one where the parasite is not transmitted, *e.g.*, near Lake Victoria to the Kenya highlands. Belief in this theory influenced the first strategy that I used to ascertain whether sickle-cell heterozygotes are relatively resistant to malaria. A laboratory had been established in Nairobi where volunteers were inoculated with *P. falciparum* to assay the efficacy of antimalarial drugs. Ethical questions related to this procedure are discussed elsewhere (Allison 2002a). Inoculation of *P. falciparum* showed that AS heterozygotes were not altogether resistant to the infection, but parasite rates and densities were significantly lower in AS than in AA individuals (Allison 1954a). It has since been recognized that acquired immunity to malaria can be long lasting and may well have contributed to our finding, because the AS and AA individuals were not matched for previous exposure to *P. falciparum* (see Allison 2002a).

My second strategy was to ascertain whether AS children are relatively resistant to naturally transmitted *P. falciparum*. Bearing in mind the epidemiological considerations summarized above, I selected for study children aged 4 months to 4 years in a rural Ugandan population in which antimalarial drugs were not used at that time (1953). To my delight, I found that high parasite counts were nearly four times as frequent in AA as in AS children (Allison 1954a). This statistically significant difference, together with the observations of Field (1949) correlating malarial mortality with high parasite densities, strongly suggested that AS children are more likely than AA children to survive in a highly malarious environment.

If the malaria hypothesis is correct, high sickle-cell frequencies would be confined to areas where malaria was hyperendemic. Another part of the research conducted in 1953 was a survey of nearly 5000 East Africans. A memorable journey took me from the Semiliki Forest of Western Uganda, where 40% of Baamba are AS, past the Tanganyikan (now Tanzanian) shore of Lake Victoria, where 35% are AS, through the highlands of Kenya and Tanganyika, where none are AS, to the coasts of Kenya and Tanganyika, where several tribes have AS frequencies of 20% (Allison 1954b). This distribution, involving diverse populations, supported the belief that an environmental factor, malaria transmission, was the principal determinant of high sickle-cell frequencies. In contrast, Lehmann (1954) and Foy *et al.* (1954) proposed that high sickle-cell frequencies in East Africa resulted from migrations of people from southern India and Arabia. This concept did not fit the observed distribution (*i.e.*, higher frequencies around Lake Victoria and in Western Uganda than at the coast) and was later disproved by the finding that the S mutation in India and Arabia is different from those in Africa (see below).

My third article was a theoretical analysis of the sickle-cell polymorphism (Allison 1954c). It was calculated that, where the AS frequency was maximal (40%), the fitness of AS must be 26% greater than that of AA to produce a stable polymorphism. In many African populations, AS frequencies are ~20%, and a 10% greater fitness of the heterozygotes suffices for a stable polymorphism. Once the sickle-cell mutation becomes established in a malarious area, its frequency can rise rapidly to approach equilibrium.

These three articles certainly aroused interest when they were published and when the observations were presented at the Cold Spring Harbor Symposium on Population Genetics (Allison 1955). The audience at this symposium had been softened up by 2 days of higher mathematics, and many were relieved to hear a straightforward message with a memorable punch line: disease is an agent of natural selection, as are competition and selective predation. Furthermore, selection through disease can maintain stable polymorphism.

My conclusions initially provoked some skepticism because of two publications. Beutler *et al.* (1955) reported that in Americans of African origin, inoculated with *P. falciparum*, parasite densities were somewhat lower in AS than in AA individuals, but the difference was not statistically significant. In such nonimmune subjects, the infections had to be terminated before reaching potentially dangerous levels, so the situation was different from that during natural infections. Foy *et al.* (1955) reported that in one group of Luo under 6 years of age, *P. falciparum* densities were significantly lower in AS than in AA children, but in another group they were not. The authors did not provide crucial data, such as whether one group had more older children (4–6 years, with acquired immunity).

During the next few years, investigators in several African countries confirmed my observations in young children. When all the observations were reviewed (Allison 1964, 2002a), they showed highly significant differences between the proportion of AA and AS children with high parasite densities and no significant heterogeneity between the observations in different populations. Furthermore, only 1 AS child was found among >100 dying of cerebral malaria, significantly fewer than expected from the AS frequencies in the populations represented. This was direct evidence that AS children are protected from a lethal form of malaria. Further confirmation has since been published, including observations made under well-controlled field conditions in The Gambia (Hill *et al.* 1991). These showed that AS children have >90% protection against severe malaria, as compared with the 76% protection of AS relative to AA children in my original report (Allison 1954a).

P. falciparum also multiplies less well in cultures of AS than in AA red blood cells under mildly anoxic conditions (reviewed by Nagel and Roth 1989). Parasitized red blood cells adhere *in vivo* to venous endothelium, so these conditions are realistic. The concept that AS heterozygotes are relatively resistant to malaria is now generally accepted and explains the distribution of the gene in Africa (Figures 1 and 2). High S gene frequencies are confined to a belt in Central Africa, south of the Sahara Desert and north of southern Africa. In the highlands of Ethiopia, East Africa, and the Cameroons, AS heterozygotes are rare. In other continents, high S frequencies are found among tribal groups in South India and in two parts of Greece. All live in areas that were intensely malarious until control was introduced (reviewed by Allison 2002a). Despite 50 years of study, involving hundreds of thousands of subjects, nobody has found an indigenous population with a high S frequency living in an area where malaria was not transmitted.

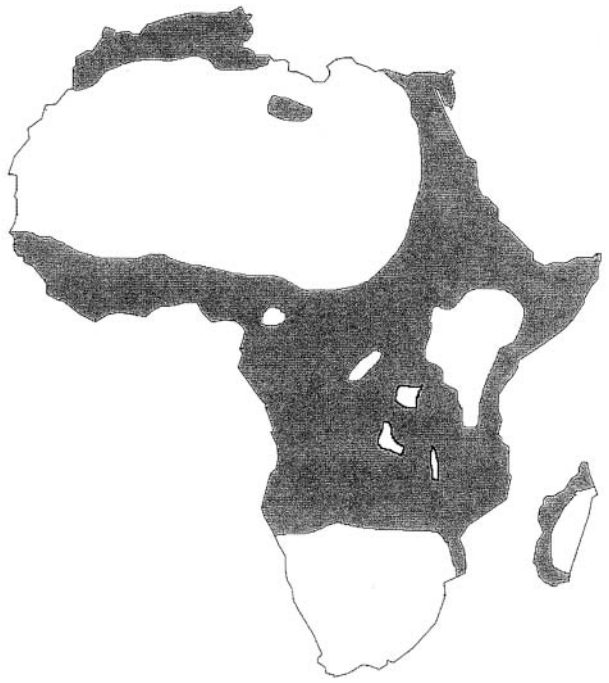

Figure 1.—The distribution of *P. parum* in Africa before malaria control was introduced (modified from Boyd 1949).

SEPARATE SICKLE-CELL MUTATIONS

The next major advance was the demonstration by Ingram (1959) that HbS differs from HbA because of a single amino-acid substitution. In the β-chain of HbA, the sixth residue is glutamic acid (negatively charged), whereas in HbS it is valine (neutral). When the triplet codes for amino acids were determined and DNA could be sequenced, the nature of the mutation itself was established. Sickle-cell disease occurs because of a substitution of thymine for adenine in the DNA codon for glutamic acid (GAG → GTG); in consequence, the β6 Glu in HbA becomes β6 Val in HbS.

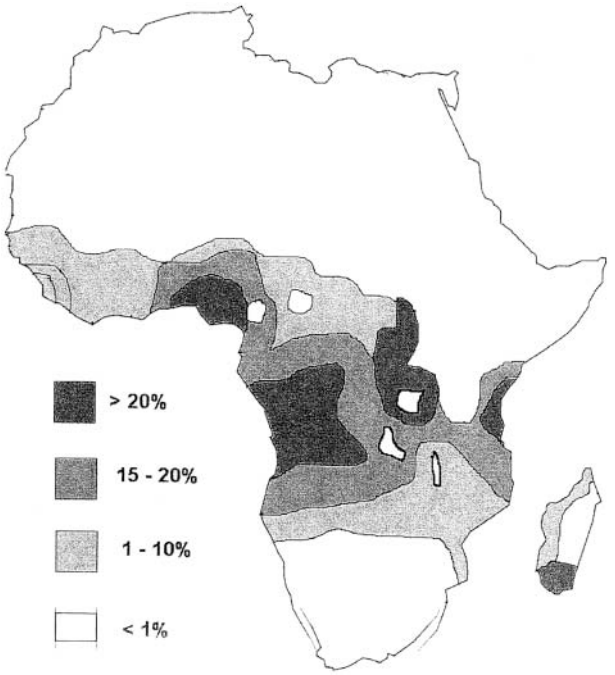

FIGURE 2.—Frequencies of sickle-cell heterozygotes in different parts of Africa.

Two mutations found in nontranscribed sequences of DNA adjacent to the β-globin gene are so close to each other that the likelihood of crossover is very small. The correlations persist through many generations [extended haplotype homozygosity (EHH)], providing a marker for population affinities and movements. Restriction endonuclease digests of the β-globin gene cluster have shown five distinct patterns associated with the sickle-cell (GAG → GTG) mutation. Four are observed in Africa (the Bantu, Benin, Senegal, and Cameroon types; LAPOUMEROULIE *et al.* 1992), and a fifth type is found in the Indian subcontinent and Arabia (LABIE *et al.* 1989). The authors cited summarize evidence that haplotype analysis in the β-globin region shows strong linkage disequilibrium over the distance indicated. This is evidence that the HbS mutation occurred independently at least five times. The high levels of AS in parts of Africa and India resulted presumably from independent selection occurring in different populations living in malarious environments.

OTHER ABNORMAL HEMOGLOBINS AND G6PD DEFICIENCY

The frequencies of abnormal hemoglobins in different populations vary greatly, but some are undoubtedly polymorphic. Three of these are β-thalassemia, with frequencies up to 10% in parts of Italy (BIANCO *et al.* 1952); HbE (β26Glu → Lys), which attains frequencies up to 55% in Thailand and other Southeast Asian countries (FLATZ 1967); and HbC (β6Glu → Lys), which attains frequencies approaching 20% in northern Ghana and Burkina-Faso (MODLANO *et al.* 2001). All of these are in malarious areas, and there is evidence that the persons with HbE and HbC have some protection against the parasite (HUTAGALUNG *et al.* 1999; MODLANO *et al.* 2001).

Deficiencies of erythrocyte glucose-6-phosphate dehydrogenase (G6PD) are polymorphic in malarious regions in African, Mediterranean, and Southeast Asian countries. Severe *falciparum* malaria is less frequent in G6PD-deficient African children than in those with normal enzymes (ALLISON and CLYDE 1961; RUWENDE *et al.* 1995).

There is little doubt that malarial selection played a major role in the distribution of all these polymorphisms. An additional question is raised by the presence of polymorphisms for HbS and another Hb mutation in the same population. Double heterozygotes for HbS and β-thalassemia, and for HbS and HbC, suffer from variant forms of sickle-cell disease, milder than SS but likely to reduce fitness before modern treatment was available. As predicted (ALLISON 1964), these variant alleles tend to be mutually exclusive in populations. There is a negative correlation between frequencies of HbS and β-thalassemia in different parts of Greece and of HbS and HbC in West Africa (see ALLISON 1964, 2002a).

Where there is no adverse interaction of mutations, as in the case of abnormal hemoglobins and G6PD deficiency, a positive correlation of these variant alleles in populations would be expected and is found (ALLISON 1964, 2002a).

GENERAL IMPLICATIONS FOR POLYMORPHISM

My articles published in 1954 showed that disease is an agent of natural selection, and many human polymorphisms are now thought to be influenced by selection through disease. Other polymorphic genes, including those for HLA-Bw53 and a CD40 ligand variant, likewise decrease susceptibility to malaria (HILL *et al.* 1991; SABETI *et al.* 2002a). All of these can be considered examples of innate resistance. The sickle-cell findings also showed that there is synergism of innate resistance and acquired immunity. The AS genotype increases chances of survival in children during the first few years of exposure to malaria; later, the powerful effects of acquired immunity overshadow those of innate resistance. The same synergism probably applies to other mechanisms of innate resistance such as Toll receptors.

These findings have implications beyond malaria. DNA sequencing has established that the human genome is highly polymorphic, and the question arises as to how many of these variations are subject to selection. Many are likely to be neutral as far as selection is concerned, as postulated by Sewall Wright and Motoo Kimura, while others are clearly subject to selection.

A framework for detecting the imprint of recent positive selection has been proposed by SABETI *et al.* (2002b).

It involves identifying haplotypes at a locus of interest (core haplotypes) and assessing the age of each core haplotype by the decay of its association with alleles at various distances from the locus. This is measured by EHH, as mentioned above. Core haplotypes that have an unusually high EHH and a high population frequency indicate the presence of a mutation that rose to prominence in the human gene pool faster than expected under neutral evolution ("selective sweep or hitchhiking"). At two loci implicated in resistance to malaria (G6PD deficiency and CD40 ligand), the core haplotypes were found to stand out and show significant evidence of selection. The authors propose that, more generally, the method could be used to scan the entire genome for evidence of recent positive selection. The demonstration 50 years ago that a polymorphic genetic locus confers resistance to malaria still provides insights into currently investigated genetic problems.

"SUCCESS HAS MANY PARENTS" (J. F. KENNEDY)

There is no more potent allergen than a new idea. The first reaction of colleagues, as Kuhn (1996) has pointed out, is to insist: "It isn't true." When that position can no longer be maintained, and a paradigm shift occurs, the second reaction is: "It isn't new." If a household name can somehow be implicated in the genesis of the idea, so much the better: it adds tone to the field.

The "malaria hypothesis" is often attributed to Haldane (1949). What actually happened is that in 1949 G. Montalenti, I. Bianco, and E. Silvestroni were analyzing the distribution of heterozygotes for β-thalassemia in Italy. They found surprisingly high frequencies (up to 10% of heterozygotes) in the Po delta region and in parts of Sardinia and Sicily. Following a meeting presentation by Haldane, Montalenti (1949) pointed out that the distribution of β-thalassemia in Italy was consistent with the hypothesis that heterozygotes might be relatively resistant to malaria. In the printed discussion, Haldane agreed with Montalenti's suggestion, and at another meeting the same year Haldane (1949) repeated the suggestion without acknowledgment of its source. However, Montalenti evidently did not take his own suggestion seriously, because, when the observations of his group on the distribution of β-thalassemia in Italy were published (Bianco *et al.* 1952), malaria was not mentioned. The concept was resurrected by Ceppellini (1955) after my presentation of observations on malaria and the sickle-cell trait at a Cold Spring Harbor Symposium (Allison 1955).

When this speculation was made by Montalenti in 1949, I was doing field work in East Africa, unaware of what was being discussed at congresses in Europe. I independently formulated the malaria hypothesis to explain the distribution of the sickle-cell trait in Kenya. The difference is that I did not abandon the idea, but went on to show that it was correct. Malaria was not mentioned by others (Foy *et al.* 1954; Lehmann 1954), who were investigating the distribution of the sickle-cell trait in East Africa as late as 1954, when my observations were published.

APPLICATION OF HUMAN GENETICS TO DEFINE A THERAPEUTIC TARGET

Now we progress to the second lesson. After the discovery of inherited variations in hemoglobins and plasma proteins, seeking polymorphisms in enzymes became fashionable. Eloise Giblett, of the King County Blood Bank, Seattle, was analyzing electrophoretically demonstrable variations in red cell adenosine deaminase (ADA). Two children were found to have no detectable ADA, and both suffered from a combined immunodeficiency affecting T- and B-lymphocytes, but with normal mental development (Giblett *et al.* 1972). The association of a rare enzyme deficiency with a rare clinical syndrome implied that they were causally related, as confirmed by subsequent research (Meuwissen *et al.* 1975).

Another inherited defect of purine metabolism is the Lesch-Nyhan syndrome (Nyhan 1975). These children lack a major enzyme of purine salvage, hypoxanthine-guanine phosphoribosyl transferase (HGPRT). They have mental retardation, spastic cerebral palsy, choreoathetosis, and self-mutilating behavior, as well as hyperuricemia and its consequences. The English authority on this syndrome during the 1970s was Richard Watts, my colleague at the Medical Research Council Clinical Research Centre, Harrow, United Kingdom. Immune functions had not been tested in Lesch-Nyhan patients, so we decided to study them and found them to be essentially normal (Allison *et al.* 1975).

These results showed that a major purine salvage pathway, mediated by HGPRT, is important for the development of the brain, but not for the responses of lymphocytes to antigenic and mitogenic stimulation. Conversely, ADA is essential for the functions of human T- and B-lymphocytes, but not for the brain. Much has been written about the mechanism by which ADA deficiency affects lymphocyte function. A likely explanation is that, in the absence of ADA, adenosine nucleotides accumulate and guanosine nucleotides are relatively depleted (Allison and Eugui 2000). This imbalance allosterically inhibits the activities of two key enzymes of purine synthesis, phosphoribosyl pyrophosphate synthetase and ribonucleotide diphosphate reductase (Figure 3). Thus, ADA deficiency results in decreased *de novo* synthesis of guanosine ribonucleotides and deoxyribonucleotides.

As Francis Crick said, "You can ignore Nature when she whispers but not when she shouts." To me, the outcomes of these genetic defects were a revelation: if one wished to produce an immunosuppressive drug, a promising strategy would be to identify an inhibitor of *de*

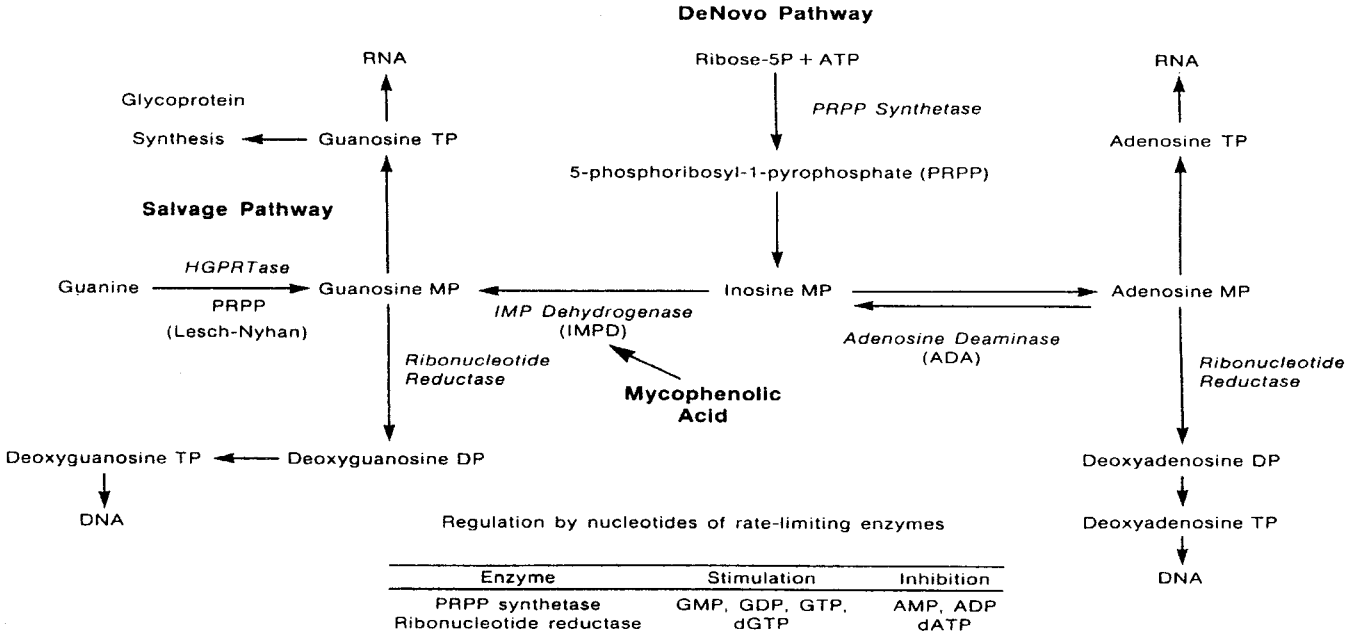

FIGURE 3.—Pathways of purine biosynthesis, showing the central position of inosine monophosphate (IMP). Mycophenolic acid inhibits IMP dehydrogenase, thereby depleting GMP, GTP, and dGTP. Two rate-limiting enzymes are activated by guanosine ribonucleotides and dGTP, but inhibited by AMP, ADP, and dATP, respectively.

novo guanosine nucleotide synthesis. The rate-limiting enzyme in this pathway is inosine-5′-monophosphate dehydrogenase (IMPDH). Why would one want to develop another immunosuppressive drug? Cyclosporin A can damage kidneys and induce hypertension, which is not an ideal profile for a drug used in renal transplantation. It was, therefore, worth exploring other strategies. At the time I was a consultant to several major pharmaceutical companies and tried to convince them that inhibiting IMPDH could lead to a useful drug. They were not interested, so the next step was delayed until 1981, when I was invited to become vice-president for research of Syntex, a pharmaceutical company in Palo Alto, California.

In 1982, Elsie Eugui and I initiated a program for comparing the immunosuppressive effects of known inhibitors of IMPDH. We avoided nucleoside analogs, which have to be phosphorylated, can inhibit DNA repair enzymes, and can produce chromosome breaks. We eventually selected mycophenolic acid (MPA), a fermentation product of *Penicillium brevicompactum* and related species (EUGUI *et al.* 1991a,b). MPA is a potent, reversible, noncompetitive inhibitor of IMPDH. Our Syntex colleague Yutaka Natsumeda cloned and expressed in *Escherichia coli* two isoforms of human IMPDH, encoded by separate genes: the widely expressed housekeeping type I isoform and the type II isoform, which is expressed in activated T- and B-lymphocytes. MPA was found to be about five times more potent as an inhibitor of the type II enzyme than as that of the type I enzyme (CARR *et al.* 1993). This was consistent with our observation that the dose of MPA required to suppress the proliferation of human T- and B-lymphocytes was about one-fifth of that required for cytostatic effects on fibroblasts and other cell types (EUGUI *et al.* 1991a). Thus, lymphocyte selectivity of MPA is achieved in two ways: by the requirement of *de novo* guanosine nucleotide synthesis for proliferation of these cells and by the greater potency of the drug on the isoform of IMPDH expressed in activated lymphocytes.

Depleting guanosine triphosphate (GTP) in lymphocytes and monocytes, mediated by MPA, was found to have another beneficial effect (ALLISON *et al.* 1993). GTP is required for the transfer of fucose and mannose, through GDP sugar intermediates, to membrane glycoproteins and glycolipids. These sugars in terminal oligosaccharides are recognized by adhesion molecules termed selectins. As a result, MPA treatment inhibits the attachment of mononuclear cells to endothelial cells. Our initial report on this mechanism of action (ALLISON *et al.* 1993) has been confirmed in several laboratories, and it has been shown that in experimental animals MPA suppresses the recruitment of lymphocytes and monocytes into grafted organs (reviewed by ALLISON 2002b). This mechanism is particularly relevant to the prevention of chronic organ graft rejection.

The prediction that MPA would have useful immunosuppressive activity was therefore confirmed, and the rest was development. An ester prodrug was shown to increase the bioavailability of MPA following oral administration. The prodrug, mycophenolate mofetil (MMF; CellCept), was found to prevent allograft rejection in several experimental animal models and in human clinical trials. The drug is now used in various

combination therapies to prevent the rejection of human kidney, liver, heart, and lung grafts (Allison and Eugui 2000). As discussed elsewhere (Allison 2002b), the drug is more effective in preventing chronic rejection than alternative therapies are. Cyclosporin A and other calcineurin inhibitors induce the production of TGFβ, which is fibrogenic in the kidneys and other organs. Rapamycin increases cholesterol and triglyceride levels, which could contribute to graft atherosclerosis and diabetes mellitus. Thus, CellCept keeps organ grafts in good functional condition for many years; this is desirable for several reasons, including the shortage of donor organs and the costs of retransplantation. Since 1995, when CellCept was introduced, the survival of organ grafts in all categories has significantly increased (Cai *et al.* 1992). CellCept is now widely used, as reflected by annual sales exceeding $1 billon.

Our genetically defined target, IMPDH, is universally recognized as a good one for the development of immunosuppressive drugs. In pharmaceutical research, as in life, imitation is the sincerest form of flattery, and several companies are exploring IMPDH inhibitors (see Sintchak and Nimmesgern 2000). Roche's main competitor, Novartis, has developed another formulation of mycophenolic acid, the ultimate compliment.

ROLE OF GENETICS IN THERAPEUTIC DEVELOPMENT

Sequencing the human genome was completed in 2003, the fiftieth anniversary of the discovery of the structure of DNA. Among the celebratory publications was a vision of the future of genomics research, including the identification of therapeutic targets (Collins *et al.* 2003). By now it is generally accepted that genetic analyses can clarify the pathogenesis of diseases and point to novel therapeutic approaches. Most large pharmaceutical companies, and many small ones, have groups exploring this field. However, according to the sage advice of Shakespeare, "thoughts are but dreams till their effects be tried." When was the concept that genetics can be used to define a therapeutic strategy first tried and shown to be true?

Several established therapies can be considered as falling under the broad umbrella of genetics, which covers a lot of biology. Genetic methods are applied to produce recombinant human erythropoietin, insulin, growth hormone, and interferons. However, recognition of the need for replacement therapy came from endocrinology and that for interferons came from virology. Enzyme replacement therapy arose out of clinical biochemistry. The development of antagonists and agonists selective for receptor subtypes now depends on cloning and expression of the target proteins. However, this is an extension of traditional receptor pharmacology. As far as I am aware, the first application of human genetics to define a major therapeutic target and to exploit it to produce a widely used drug was our program on IMPDH and mycophenolate mofetil. The program was initiated in 1982, and CellCept was approved by the Food and Drug Administration in 1995.

Genetic methods have since been used in other ways to identify therapeutic targets. A spectacular success was the development of an inhibitor of the Bcr-Abl tyrosine kinase for treatment of chronic myeloid leukemia (CML; Druker 2003). Present in ~95% of patients with CML, Bcr-Abl has been shown to be a leukemogenic oncogene in experimental animals. It functions as a constitutionally activated tyrosine kinase, and this function is required for transformation by Bcr-Abl. A small-molecule inhibitor of Bcr-Abl, tyrosine kinase (imatinib-Gleevec), proved to be an effective therapeutic agent in CML and some other malignancies. Mycophenolate mofetil and imatinib are proof in principle not only for the concept of molecular targeted therapy, but also for the application of genetics to identify molecular targets. Sadly, resistance to Gleevec eventually develops in many patients. Happily, resistance to CellCept rarely, if ever, occurs.

The grand challenge for the future presented by Collins *et al.* (2003) is certainly a splendid vision. Those who are skeptical about visions can derive comfort from the knowledge that it is more than a dream: genetics has already been applied to develop widely used therapeutic agents. During the coming decades, other major developments in this field are expected.

"IF YOU CAN DREAM—BUT NOT MAKE DREAMS YOUR MASTER . . ." (KIPLING)

My first lesson as a research scientist was not to become attached to pet ideas: they are fun to play with but need careful evaluation. An investigator without ideas resembles champagne without bubbles; however, most ideas, like bubbles, are evanescent. The majority of the ideas that survive result in potboilers, which have their place in sustaining the advancement of science. Very few ideas are good enough to result in even a minor paradigm shift or to open up a field of investigation. Seeing that happen to one's own brainchildren is the ultimate thrill for a research worker. It is something to have had the first word on such a topic; having the last word is impossible, but with adequate documentation one can have the last word on the first.

AN OCCASIONAL BACKWARD GLANCE

A cautionary tale was finding that few investigators read articles more than 5 years old, a practice encouraged by electronic retrieval of publications. However, some scientists still care about how their fields opened up, and a few are even interested in the history of branches of science other than their own. Recapitulating the history of sickle-cell research is currently being

used as an exercise in science education and is reported to increase student's understanding of the nature of science (Howe 2003). Good science education is a national need, and it is gratifying to know that contemporary students learn this small part of the history of science and are stimulated by it to think for themselves. In view of the widespread collective amnesia of the scientific community, it is remarkable that articles are still read and quoted 50 years after publication.

When I lecture on the application of genetics to identify therapeutic targets, students are enthusiastic. The achievements in that field are already impressive, and the promise is even greater. The promise will be realized for the most part by scientists who are now beginning their careers, and some of them will cast a backward glance at how it all began. Looking back in science carries no penalties, as it did for Orpheus and Lot's wife.

Colleagues who contributed to the research reviewed here are too numerous to list. They and I know where they fit into the stories. The collaboration and support of my wife, Elsie Eugui, throughout the development of MMF is gratefully acknowledged. Among Syntex colleagues who participated in that program, two deserve special mention: Peter Nelson, who synthesized derivatives of MPA, and Yutaka Natsumeda, for assaying the effects of MPA on isoforms of IMPDH. Thank you all for contributing to a successful outcome.

LITERATURE CITED

Allison, A. C., 1954a Protection afforded by the sickle-cell trait against subtertian malaria infection. Br. Med. J. **1:** 290–294.

Allison, A. C., 1954b The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria. Trans. R. Soc. Trop. Med. Hyg. **48:** 312–318.

Allison, A. C., 1954c Notes on sickle-cell polymorphism. Ann. Hum. Genet. **19:** 39–57.

Allison, A. C., 1955 Aspects of polymorphism in man. Cold Spring Harbor Symp. Quant. Biol. **20:** 239–251.

Allison, A. C., 1964 Polymorphism and natural selection in human populations. Cold Spring Harbor Symp. Quant. Biol. **29:** 137–149.

Allison, A. C., 2002a The discovery of resistance to malaria of sickle-cell heterozygotes. Biochem. Mol. Biol. Edu. **30:** 279–287.

Allison, A. C., 2002b Mechanisms of action of mycophenolate mofetil in preventing chronic rejection. Transplant. Proc. **34:** 2863–2866.

Allison, A. C., and D. F. Clyde, 1961 Malaria in African children deficient in glucose 6 phosphate dehydrogenase. Br. Med. J. **1:** 1346–1349.

Allison, A. C., and E. M. Eugui, 2000 Mycophenolate mofetil and its mechanisms of action. Immunopharmacology **47:** 85–118.

Allison, A. C., E. W. Ikin, A. E. Mourant and A. B. Raper, 1952 Blood groups and other genetic traits in East African tribes. J. R. Anthropol. Inst. **82:** 55–60.

Allison, A. C., T. Hovi, R. W. E. Watts and A. D. B. Webster, 1975 Immunological observations on patients with the Lesch-Nyhan syndrome, and on the role of *de novo* purine synthesis in lymphocyte transformation. Lancet **2:** 1179–1183.

Allison, A. C., W. J. Kowalski and C. J. Muller, 1993 Mycophenolic acid and brequinar, inhibitors of purine and pyrimidine synthesis, block the glycosylation of adhesion molecules. Transplant. Proc. **25** (Suppl. 2): 67–70.

Beet, E. A., 1949 The genetics of the sickle-cell trait in a Bantu tribe. Ann. Eugen. **14:** 279–284.

Beutler, E., R. J. Dern and C. L. Flanagan, 1955 Effect of sickle-cell trait on resistance to malaria. Br. Med. J. **1:** 1189–1190.

Bianco, I., G. Montalenti, E. Silvestroni and M. Siniscalco, 1952 Further data on genetics of microcythaemia or thalassaemia minor and Cooley's disease or thalassaemia major. Ann. Eugen. **16:** 299–315.

Boyd, M. F., 1949 *Malariology*. W. B. Saunders, Philadelphia.

Cai, J., D. W. Gjertson and P. I. Terasaki, 1992 Maintenance immunosuppression and graft half life, pp. 359–366 in *Clinical Transplants 2002*, edited by J. M. Cecka and P. I. Terasaki. Immunogenetics Center, Los Angeles.

Carr, S. F., E. Papp, J. C. Wu and Y. Natsumeda, 1993 Characterization of human type I and type II IMP dehydrogenases. J. Biol. Chem. **268:** 27286–27290.

Ceppellini, R., 1955 Discussion of "Aspects of Polymorphism in Man." Cold Spring Harbor Symp. Quant. Biol. **20:** 251–255.

Collins, F. S., E. D. Green, A. E. Guttmacher and M. S. Guyer, 2003 A vision for the future of genomics research. Nature **422:** 835–847.

Diggs, L. W., G. F. Ahmann and S. Bibb, 1933 The incidence and significance of the sickle cell trait. Ann. Intern. Med. **7:** 769–778.

Druker, B. J., 2003 Imatinib alone and in combination for myeloid leukemia. Semin. Hematol. **40:** 50–58.

Eugui, E. M., S. Almquist, C. D. Muller and A. C. Allison, 1991a Lymphocyte-selective cytostatic and immunosuppressive effects of mycophenolic acid *in vitro*: the role of deoxyguanosine nucleotide depletion. Scand. J. Immunol. **33:** 161–173.

Eugui, E. M., A. Mirkovich and A. C. Allison, 1991b Lymphocyte-selective and immunosuppressive effects on mycophenolic acid in mice. Scand. J. Immunol. **33:** 175–183.

Field, J. W., 1949 Blood examination and prognosis in acute *falciparum* malaria. Trans. R. Soc. Trop. Med. Hyg. **48:** 312–318.

Flatz, G., 1967 Hemoglobin E: distribution and population dynamics. Humangenetik **3:** 189–234.

Foy, H., A. Kondi, G. L. Timms, W. Brass and F. Bushra, 1954 The variability of sickle-cell rates in Kenya and the Southern Sudan. Br. Med. J. **1:** 1189–1190.

Foy, H., W. Brass, R. A. Moore, G. L. Timms, A. Kondi *et al.*, 1955 Two surveys to investigate the relation of the sickle-cell trait and malaria. Br. Med. J. **2:** 1116–1119.

Giblett, E. B., J. E. Anderson, F. Cohen, B. Pollara and H. J. Meuwissen, 1972 Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. Lancet **2:** 1067–1069.

Greenwood, B., K. Marsh and R. Snow, 1991 Why do some African children develop severe malaria? Parasitol. Today **7:** 277–281.

Haldane, J. B. S., 1949 The rate of mutation of human genes. Proc. Int. Congr. Genet. Hered. **35** (Suppl.): 267–273.

Herrick, J. B., 1910 Peculiar elongated and sickle-shaped red corpuscles in a case of severe anemia. J. Am. Med. Assoc. **261:** 266–271.

Hill, A. V. S., C. E. M. Allsopp, D. Kwiatkowski, N. M. Anstey, P. Twumasi *et al.*, 1991 Common West African HLA antigens are associated with protection from severe malaria. Nature **352:** 595–600.

Howe, E., 2003 Recapitulating the history of sickle-cell anemia research: improving students' nature of science views explicitly and reflectively, pp. 22–30 in *Proceedings of the Seventh International History, Philosophy, and Science Teaching Group Meeting*, edited by D. Metz. Winnipeg, Manitoba, Canada.

Hutagalung, R., P. Wilairtana, S. Loorreesuwan, G. M. Brittenham, M. Aikawa *et al.*, 1999 Influence of haemoglobin E trait on the severity of *falciparum* malaria. J. Infect. Dis. **129:** 283–286.

Ingram, V. M., 1959 Abnormal human hemoglobins. III. The chemical difference between normal and sickle-cell hemoglobins. Biochim. Biophys. Acta **36:** 402–411.

Kuhn, T. S., 1996 *The Structure of Scientific Revolutions*. The University of Chicago Press, Chicago.

Labie, D., R. Srinivas, O. Dunda, C. Dode, C. Lapoumeroulie *et al.*, 1989 Haplotypes in tribal Indians bearing the sickle gene: evidence for the unicentric origin of the β_S mutation and the unicentric origin of tribal populations in India. Hum. Biol. **61:** 479–491.

Lapoumeroulie, C., O. Dunda, R. Durocq, G. Trabuchet, M. Mony-Lobe *et al.*, 1992 A novel sickle-cell mutation of yet another origin in Africa: the Cameroon type. Hum. Genet. **89:** 333–337.

Lehmann, H., 1954 Distribution of the sickle-cell gene. Eugen. Rev. **46:** 101–121.

Meuwissen, H. J., R. J. Pickering, B. Pollara and I. H. Porter (Editors), 1975 *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency: A Molecular Defect*. Academic Press, New York.

Modlano, D., G. Luoni, B. S. Sirima, J. Simpore, F. Verra *et al.*, 2001 Haemoglobin C protects against clinical *P. falciparum* malaria. Nature **414:** 305–308.

Montalenti, G., 1949 Comment on Haldane, J. B. S. Disease and evolution. Ric. Sci. **19** (Suppl.): 333–334.

Nagel, R. L., and E. F. J. Roth, 1989 Malaria and red cell genetic defects. Blood **74:** 1213–1221.

Neel, J. V., 1949 The inheritance of sickle-cell anemia. Science **110:** 64–66.

Nyhan, W. L., 1975 The Lesch-Nyhan syndrome, pp. 59–87 in *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency: A Molecular Defect*, edited by H. J. Meuwissen, R. J. Pickering, B. Pollara and I. H. Porter. Academic Press, New York.

Pauling, L., 1994 Preface, pp. xvii–xix in *Sickle-Cell Disease: Basic Principles and Clinical Practice*, edited by S. H. Embury, S. P. Hebbel, N. Mohandas and M. S. Steinberg. Raven Press, New York.

Pauling, L., H. A. Itano, S. J. Singer and I. C. Wells, 1949 Sickle-cell anemia: a molecular disease. Science **110:** 543–547.

Ruwende, C., S. C. Khoo, R. W. Snow, S. N. R. Yates, S. Kwiatkowski *et al.*, 1995 Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to falciparum maliria. Nature **378:** 246–249.

Sabeti, P., S. Usen, S. Farhadian, M. Jallow, T. Doherty *et al.*, 2002a CD40L association with protection from severe malaria. Genes Immun. **3:** 286–291.

Sabeti, P. C., D. E. Reich, J. M. Higgins, H. Z. P. Levine, D. Richter *et al.*, 2002b Detecting recent positive selection in the human genome from haplotype structure. Nature **419:** 832–837.

Sintchak, M. D., and E. Nimmesgern, 2000 The structure of inosine-5′-monophosphate dehydrogenase and the design of novel inhibitors. Immunopharmacology **47:** 163–184.

Instructor's note: pages 5,6 and 7 describe how the insight that sickle cell trait protects against malaria led to new clinical applications. Other red blood cell mutations that affect immunity from disease were discovered. Those mutations gave the author the idea to test the role of certain enzymes and their inhibitors involved in immune responses. One inhibitor led to the development of a new medication tht is useful in organ transplantation. This is an example of the indirect way that evolutionary medical hypotheses can lead to new treatments of disease.