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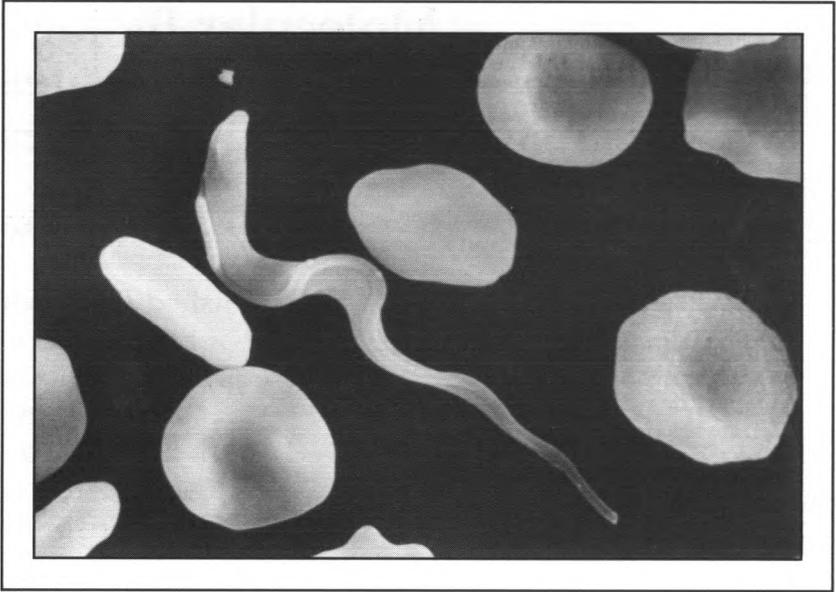
Molecular Biology of Parasites Causing Tropical Diseases

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An African trypanosome among red blood cells.

Molecular Biology
of Parasites
Causing Tropical Diseases

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Thank you very much, President Rawlings. It is an honor and a pleasure to present this year's Presidential Lecture. I also would like to thank all of you in the audience for coming inside on this fine winter afternoon to hear about the tropics—and specifically about diseases that are found in the tropics.

As we speak, more than 5 billion people are alive on the face of this earth. Most of these 5 billion people live in tropical areas of the world. Only about 2.5 million people, or 1 in 2000, live here in the state of Iowa. Yet, as we shall see, by at least one measuring stick the extent of health care available to those of us in Iowa is equivalent to that received by the rest of the world combined.

I'd like to begin this year's Presidential Lecture by presenting some startling statistics on world health and biomedical research. We'll also spend a few minutes reviewing how one of the most devastating infectious diseases in the history of the world, smallpox, was completely eradicated about 10 years ago. Using smallpox as a background, we'll then look at three of the most serious infectious diseases in the world today and examine the possibilities for eliminating them as well. We'll talk about the organisms that cause these three diseases, how they evade the immune defenses of our body, and what the prospects for a vaccine are. Finally, we'll finish by turning to the excellent Stanley collection of African Art in The University of Iowa Art Museum for a look at how disease has influenced the development of art in tropical countries.

Some Facts and Figures
About Research in Tropical Medicine

The 40 poorest countries of the world are home to about 3 billion people, according to statistics compiled by UNICEF and the World Bank. These countries, as defined by annual income per capita, are mainly in the tropical areas of Africa, Asia and Latin America. People living in these countries have an average annual income of $270 per year. Their literacy rate is 30%, a percent that has actually increased substantially during the past generation. Their average life expectancy at birth is 48 years—compared to nearly 80 years in this country and Western Europe. Their infant mortality is 130 deaths/1000 births. Yet, despite this low life expectancy and high infant death rate, the annual growth rate in these countries is alarmingly high— 2.5%. Some countries, such as Kenya in East Africa and Niger in West Africa, are estimated to have an annual growth rate of nearly 4%. As an illustration of the political and economic strain imposed by this level of growth rate, Kenya was a net exporter of foodstuff 25 years ago; now it is an net importer of food.

Associated with this abysmal income of $270/year and high population growth rate are enormous problems of heath care and health care delivery. The governments of developing countries simply don't have the resources to provide health service to their citizens nor the means to get medical attention to the rural areas that need it most. Yet it is these tropical areas of the world where infectious diseases are most prevalent.

A few years ago the World Health Organization drew up a list of the major diseases of the world, which is presented in TABLE 1.

TABLE 1
The Major Diseases of the World
(Source: World Health Organization)

	# people infected
1. Malaria	800 million
2. Schistosomiasis	200 million
3. Leishmaniasis	200 million
4. Filariasis	
lymphatic	250 million
river blindness	30 million
5. Trypanosomiasis	
Chagas' disease (Latin America)	12 million
sleeping sickness (Africa)	1 million

About 800 million people are currently infected with *malaria* parasites. With the exception of bacterial infections and diarrheal diseases which can be reduced or prevented by proper hygiene and nutrition, malaria is the most prevalent disease in the world today. It is estimated that 1 million infants die each year from malaria or its complications. Yet an individual who survives repeated bouts of malaria infections as a child acquires some immunity against malaria in adulthood. Such an individual may still experience sporadic malaria attacks but the severity of the attack is greatly reduced. This fact suggests that it might be possible to develop a vaccine against malaria; we will have more to say about this possibility later.

Schistosomiasis, leishmaniasis and *filariasis* are each diseases that affect about 200-300 million people worldwide. Not as many people succumb to these infections as die of malaria but they contribute significantly to malaise and a poor quality of life. They also tax the body's immune system and render people more susceptible to bacterial infections and other secondary infections. *Trypanosomiasis* appears in two forms that affect far fewer people but whose impact can be just as severe. About 12 million people in Latin America have Chagas' disease—a disease for which there is no known cure if the initial infection is not detected early. In Africa another trypanosome species cause sleeping sickness—a fatal disease, if left untreated, that relatively few people now acquire but whose impact is enormous because domestic animals such as cattle, sheep, and pigs are particularly susceptible.

Each of these five infectious diseases is a parasitic disease. They are all caused by parasites that are transmitted to humans by insects, or in the case of schistosomiasis from fresh water snails. Furthermore, the diseases are often additive. Many people in developing countries are co-infected with more than one of the parasites. They may experience repeated bouts of malaria while, at the same time, they endure severe itching and eye disease from river blindness, and only partial kidney function due to schistosomiasis. Under these conditions it is difficult to be an active, productive and happy member of society—especially if the economy of your country is such that no job is available to you and no medical help is present for overcoming the infections.

Despite the enormous impact of these diseases on a large segment of the world's population, very little research money is devoted to their study. TABLE 2 shows the amount of money that the National

Institutes of Health (N.I.H.), the main granting agency of the United States government in the area of biomedical research, devoted to its 1988 extramural program for the study of the five main parasitic diseases.

TABLE 2
N.I.H. Extramural Funding in Tropical Medicine in 1988

DISEASE	# of people infected (millions)	$ (millions)
malaria	800	5
schistosomiasis	200	6
filariasis	200	1
trypanosomiasis	13	8
ameobiasis	500	<1
ascariasis	>1000	<1
cancer	10 (USA)	1,100
AIDS	1 (USA)	205

Only $5 million were extended in grants and contracts by N.I.H. to biomedical researchers studying malaria, a disease that affects 800 million people worldwide. That is less than 1 penny per malaria-infected person. Likewise, very little research was devoted to schistosomiasis and filariasis, diseases that affect millions of people. More money was actually spent on research on trypanosomiasis than any other parasitic disease, even though many fewer people are directly affected. This is because, as discussed below, some of the genes in trypanosomes undergo very interesting duplications and rearrangements that are potentially significant in understanding specific events in human cancer cells and in cells of the immune system. Less than $1 million dollars was spent on research on amoeba and ascaris, parasites that infect a billion or more people each year.

In contrast, N.I.H. awarded $1.1 billion in grants and contracts in 1988 to researchers studying cancer-related problems. If one assumes that 10 million people either had, or were recovering from, cancer in 1988, that value amounts to about $110/cancer patient. Even more money per patient was devoted to AIDS research. For each person thought to be infected with the AIDS virus in this country, over $200 was spent on AIDS research. Furthermore, these figures are only the amounts spent on government-sponsored laboratory research; they

do not include the cost of treatment, hospitalization and out-patient care—luxuries that are often not available in developing countries.

It should be stressed that the comparisons in TABLE 2 are not intended to malign research on cancer and AIDS. Cancer and heart disease are the two leading causes of death in this country and should be emphasized in our government-sponsored research effort. AIDS is, likewise, a devastating disease of worldwide significance and every effort must be made to combat it. Nevertheless, most people of the world do not die of cancer, heart disease or AIDS. Instead, they succumb to parasitic diseases or are debilitated by parasitic infections and become more susceptible to bacterial infections. In the 40 poorest countries of the world, over 70% of the people die of infectious diseases; in the United States less than 10% die of infections. However, developing countries do not have the financial resources or trained personnel to support infectious disease research programs. Biomedical research is notoriously expensive and inefficient, and governments in endemic areas for tropical diseases simply don't have the resources to sponsor basic biomedical research. Someone else must support this kind of research activity if it is to be conducted.

This is not to say that developing countries are uninterested in biomedical research. To the contrary, they are very interested. Chagas' disease is so important in Brazil that its paper currency contains a drawing of the life cycle of *Trypanosoma cruzi*, the parasite which causes the disease, and a picture of Carlos Chagas, the scientist who first described the disease. Brazil and many other tropical countries reserve a much larger fraction of their annual budget for health-related programs than does the United States. But the amount that most of these countries can devote to biomedical research is nowhere near the research and development expenditure of even a mid-sized pharmaceutical company in the United States. As an aside, it is interesting to note that while Brazil places pictures of its famous scientists on its currency, in this country we grace our paper currency with pictures of our politicians. On this particular point Brazil may have a better way of doing things than we do.

In developed countries such as the United States an alternative to government-sponsored research is private industry. For example, in the United States, Europe and Japan, a great deal of very high-quality biomedical research is conducted by pharmaceutical companies. However, private industry is interested only in those drugs or

vaccines that customers can pay for. The cost of developing a new drug or vaccine is enormous and the sale of the product has to be used to recover the cost of its development. Many recent drugs or vaccines developed by the pharmaceutical industry have cost a company as much as $100 million to get to the market place. These companies are understandably not interested in spending $100 million to develop a new drug intended for people or governments who cannot afford to buy it. As a result, there has been only one genuinely new, practical drug against a tropical disease introduced in the past 50 years. That drug, ivermectin, was first developed for veterinary use in the United States and later found to be safe and partially effective against human onchocerciasis.

Another obvious source of support for tropical disease research is the World Health Organization (W.H.O.), the arm of the United Nations responsible for stimulating international health-related activities. W.H.O. does indeed foster research on tropical diseases. However, the total operating budget for W.H.O. in this fiscal year is $250 million. This budget supports W.H.O.'s full range of activities, from support of high-tech laboratory research to epidemological studies and vaccine distribution in remote locations. However, since the mission of W.H.O. is directed toward practical assistance of immediate medical problems in developing countries, very little of its money is available for basic research. By way of comparison, the operating budget for University of Iowa Hospitals this year is $240 million dollars — nearly the same as the entire budget of W.H.O! I find this to be a remarkable comparison. The operating budget for a regional hospital here in the Midwest — even considering that it is a very good one — is virtually the same as the budget of the entire W.H.O., the largest international agency to which a developing country's government can turn in time of medical need.

The Complete Eradication of Smallpox from the World

Despite its financial and political limitations the W.H.O. managed to organize and successfully accomplish what is probably the most remarkable medical achievement ever carried out — the total eradication of smallpox from the earth. In 1967, when the W.H.O smallpox vaccination program got underway, there were estimated to be 10 million new smallpox cases in the world each year. Through a tremendous organizational effort thousands of small vaccination teams scoured the world for the next 10 years, vaccinating (sometimes

against their will) millions of people in India, Asia, Africa and Latin America who were likely to be exposed to smallpox. As a result, in December,1977, the last known individual with naturally-occurring smallpox, Mr. Ali Maow Maalin, a 23-year-old cook, was treated in Somalia in northeastern Africa.

Ironically, the elimination of smallpox during the 1960's and 1970's was paralleled by a striking increase in the incidence of malaria. During this time malaria was treated with several drugs derived from quinine—a compound discovered to be effective against malaria at the turn of the century. The most popular of these anti-malarial drugs was, and is, chloroquine. But in 1967 a chloroquine-resistant strain of the malaria parasite emerged for the first time and these resistant strains are now found throughout the entire tropics. Thus, the elimination of one disease was accompanied by the loss of an effective control measure against another disease. Just to emphasize that point, in 1967 there were 17 cases of malaria reported in Ceylon, now called Sri Lanka. Ten years later a malaria epidemic struck millions of people in Ceylon.

In preparation for a more detailed discussion of tropical diseases and what can be done to try to eliminate them, it is useful to look briefly at the history of smallpox eradication. Smallpox was caused by a virus that was transmitted directly from person to person in small droplets discharged from the mouth or nose. It was a very infectious disease whose prevalence, nevertheless, seems to have been rather cyclic down through history. Smallpox probably existed during ancient Egyptian times. The mummy of Ramses V, who died of disease in 1160 B.C., contains preserved skin that is pock-marked with what may be smallpox pustules. Curiously, however, smallpox is not mentioned in either the old or new testaments of the Bible. Likewise, the Roman and Greek literature of that time does not allude to smallpox. Thus, it seems not to have been prevalent during Biblical times. The middle ages, however, —1300 years later—were accompanied by some devastating epidemics of smallpox. In the year 1365 it is estimated that one-third of the population of England died of smallpox. Then just a few years later another even worse smallpox epidemic struck, killing this time as many as half of the remaining occupants of many villages.

Epidemics as desperate as these understandably stimulated intense efforts to combat the disease. The ancient Chinese were probably the

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first to discover that if pus from smallpox sores was gently scratched into the skin of a another person, that person acquired a much milder case of smallpox if he was lucky. Then in 1796, an English physician, Edward Jenner, conducted a real experiment in which he inoculated material from a dairymaid's mild cowpox lesion into the arm of an 8-year-old boy and exposed the boy to smallpox. The boy did not get smallpox and Jenner correctly concluded that he was immune. Jenner may not have been the first person to conduct this experiment—it's not known for sure—but he was the first to coin the term vaccination.

Cowpox is a disease of cattle that is caused by a virus very similar to the human smallpox virus. Yet the cowpox virus seldom causes serious lesions in humans. Vaccinia virus is still another member of this pox family of viruses that infects cattle and is even less dangerous to humans. Nearly two centuries after Jenner's discovery, W.H.O.'s campaign to stamp out smallpox involved scratching freeze-dried preparations of vaccinia virus under the skin of millions of people. By this time a great deal of experience and information was available on smallpox vaccination. This eradication effort was completely success-ful because the human body is the only place where the virus can reside for longer than a few minutes and it can only be transmitted through close contact between people. Thus, by vaccinating everyone in the vicinity of a smallpox patient, transfer of the virus from one individual to another could be interrupted. In addition, the vaccine was very easy to transport and to administer. Furthermore, since the vaccinia virus is isolated from cows, it could be readily produced in the countries where the vaccine was needed. Just one case of smallpox in the world has been confirmed since 1977, and that was a laboratory-derived infection in Britain in 1978. (The director of that laboratory subsequently committed suicide.)

Using the experiences gained from smallpox vaccination, let's now examine three of the tropical diseases that we discussed earlier—*sleeping sickness, malaria* and *river blindness*. In each case we will look at some general features of the disease and then explore the scientific questions posed by the parasite that causes the disease. As we shall see, vaccination against these diseases is not yet possible and will be much more complicated than against smallpox.

The Changing Coats of African Trypanosomes

My laboratory here at the University of Iowa has been studying sleeping sickness, or African trypanosomiasis, for nearly 10 years. It

is a disease whose effects I first saw as a Peace Corps Volunteer in Ghana, West Africa, shortly after graduating from undergraduate school. My wife, Linda, and I were teachers for two years in a rain forest region of Ghana where there were no milk products and very little meat because of this disease. Sleeping sickness is caused by a protozoan (single-celled) parasite called the trypanosome. Both humans and animals can be infected by the parasite and acquire the disease. Trypanosomes are transmitted by tsetse flies and occur wherever tsetse flies are found. The region most affected is equatorial Africa—a geographical area the size of the United States and Western Europe combined. More than 250 million people live in this region of Africa. More than 20 independent countries are affected. At least some of these countries will probably never be economically and nutritionally self-sufficient until this disease is somehow controlled. Although the disease's greatest impact nowadays is on domestic livestock, there have been serious epidemics of the human disease in the past. At the beginning of this century a major epidemic of sleeping sickness in the present-day country of Uganda is estimated to have wiped out half of the population living on the shores of Lake Victoria. Since then, no major epidemics have occurred although the threat of one remains. Nevertheless, the main consequence of the disease today is that it seriously hampers livestock raising in an area the size of the United States and Western Europe—and on a continent where nutrition is often substandard.

African trypanosomes have a simple life cycle. They spend part of their time in tsetse flies and the rest of their time in the mammalian bloodstream. When a tsetse fly takes in a blood meal from an infected mammal, the ingested bloodstream trypanosomes establish themselves in the midgut of the fly and undergo a variety of structural and metabolic changes. After about two weeks in the midgut, they migrate to the fly's salivary glands and wait there until the fly bites another mammal. When the fly bites, the trypanosomes are injected into the new animal's bloodstream where they begin to multiply and divide. While in the bloodstream they are in constant contact with their host's immune system which attempts to destroy them. They have evolved, however, a very clever way to evade the antibodies raised against them and can live in the bloodstream for many months, even years. Eventually, by a mechanism not understood, the trypanosomes enter their host's central nervous system and go to the brain. The infected host soon becomes comatose and dies—hence, the name sleeping sickness.

Several drugs exist for treating trypanosome infections. The two drugs that must be used after the parasites have entered the central nervous system are tryparsamide and melarsoprol. Both drugs are very toxic and must be administered intravenously. Their active ingredient is arsenic. To use such drugs is clearly a last-ditch resort because the idea is to try to give enough arsenic to poison the parasite without killing the patient. Such treatment with arsenic-laced drugs, even if it sometimes works in humans, is not suitable for hundreds of thousands of domestic livestock. At the moment there is no treatment against trypanosomes that can be used on a large scale in animals. A new drug, dimethyfluorornithine, that crosses the "blood-brain barrier" is available but must be used in too large amounts to be practical. There is almost no research activity devoted to trying to identify less toxic and more effective drugs against trypanosomes because, again, pharmaceutical companies see no chance for recovering the cost of identifying and developing such drugs.

Normally, when a pathogen such as an influenza virus enters our bloodstream, our immune system acts to destroy that foreign organism within a few days or weeks. When trypanosomes invade the bloodstream, however, they manage to avoid this immune response, as illustrated in FIGURE 1.
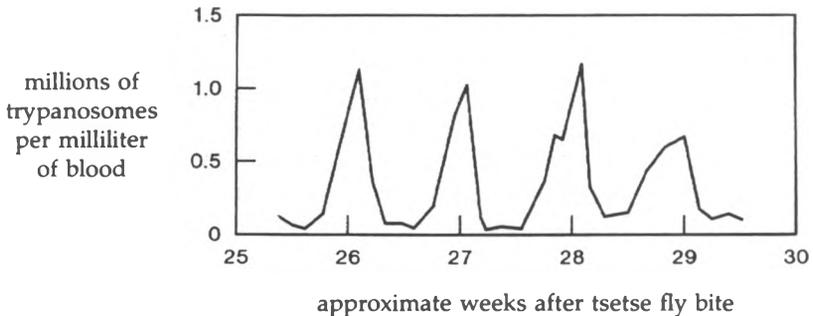

approximate weeks after tsetse fly bite

FIGURE 1 is taken from an article in the British medical literature of 1910 [R. Ross & D. Tompson (1910) Proc. R. Soc. Lond. Ser. B 82:411-415]. Sir Ronald Ross, one of the co-authors, received the second Nobel Prize ever given in Medicine (1902) for demonstrating that mosquitoes were the carriers of the malaria parasite.

As a brief historical aside, it is worth mentioning that tropical medicine research and parasitology experienced a golden age during the first decade of this century. Back then, parasitology was the most exciting area of biomedical research for a young student to enter — rather like molecular biology has been in recent times. Many important parasitological discoveries were made by medical personnel trained in Europe who spent time in India, Asia and Africa. Three Nobel Prizes were conferred on parasitologists over a period of 7 years — in 1902 to Ronald Ross, in 1907 to Charles Alphonse Laveran for the discovery of the malaria parasite and in 1908 to Paul Erlich for contributions to parasite immunology and pharmacology. It was an exciting time for tropical medicine research. The subject then went through a quieter period and only in the past 10 years, as molecular biology entered the field, has it approached again the scientific excitement that it generated at the turn of the century.

The 1910 article from which FIGURE 1 was taken describes a case study by Ross and his associate on a patient who had acquired a trypanosome infection in the former British colony of Rhodesia, now the independent country of Zimbabwe. They determined the number of trypanosomes that were in blood samples taken from the patient at various times and discovered that the infection was characterized by successive waves of parasitemia. On one day there would be many trypanosomes in the blood and then a few days later there would be only a few trypanosomes. This low level of parasites would be followed by another high level a few days later, and so on. In a remarkable stretch of imagination for the time, they correctly reasoned that the trypanosomes multiplied until the immune system mounted an effective response which killed most of the parasites, but a few organisms were able to escape this immune reaction and give rise to the next population increase. This alternation between high and low numbers of trypanosomes in the bloodstream continued until the patient died.

An understanding of trypanosome infections did not advance much further until the mid-1970's. By that time Keith Vickerman and his associates in Scotland had discovered that the surface of each trypanosome is covered with about 10 million copies of a single protein. It is this protein that the immune system recognizes as foreign and mounts a response against. Then, in 1975 George Cross and his associates in Cambridge, England, did a very interesting experiment. They infected a single experimental rabbit with a single

trypanosome clone and collected trypanosomes from the blood at various times during the infection. They used biochemical procedures to remove the surface protein from the collected trypanosomes and to analyze its structure. They found that trypanosomes collected at different times during the infection possessed a different protein on their surface. This finding immediately suggested an explanation for the cyclic numbers of trypanosomes that Ross had observed 65 years earlier. It suggested that the antibodies of the host immune system are raised against the main surface protein of a given trypanosome population. This initial immune response destroys most of the trypanosomes, but before all of the parasites are eliminated, one or more trypanosomes switches to the production of another protein on its surface. These switched trypanosomes multiply and give rise to a second population with this new protein on their surface. The immune system must raise a new set of antibodies specific for this new protein. But, again, before all of the trypanosomes are destroyed by the new antibodies, still other parasites switch to the expression of a third protein on their surface. Thus, the occasional switching of the surface protein by individual trypanosomes, followed by multiplication of the switched parasites, allows the trypanosome population as a whole to stay "one step ahead" of the immune system's effort to destroy it. It is as though a person wearing a red coat disguises himself by taking off the red coat and putting on a green coat, then later switches to a yellow coat, and so on.

Laboratory experiments have shown that an individual trypanosome has the potential to make over 100 switches to different surface proteins. A trypanosome may have, as we shall see, the capacity to produce a virtually infinite number of different proteins on its surface, each of which is immunologically distinct from the previous one. The scientific question that my laboratory, and other laboratories around the world, is trying to answer is the following: how can an organism with a limited amount of genetic material sequentially produce a very large number of unique proteins on its surface in its quest to continually evade the immune system?

As a result of much hard work by many students and post-doctoral fellows in my laboratory, and in other laboratories, we now know much of the answer to this question, but we are still missing a crucial piece of information. Experimentally, we address the question by using recombinant DNA techniques. We isolate messenger RNA (mRNA) molecules from a population of trypanosomes that express a

specific protein on its surface. Using a series of enzyme-catalyzed steps in the test tube, we convert these RNA molecules to complementary DNA (cDNA) molecules and attach these cDNAs to a bacterial plasmid DNA molecule. The resultant "recombinant" DNAs are then put back into laboratory bacterial cells. These bacterial cells become factories for producing large amounts of "cloned" trypanosome genes. It is relatively straightforward, using microbiological and immunological techniques, to identify the individual bacterial cells that contain the trypanosome gene for the surface protein that periodically switches. When this gene is identified, it can be used to determine the amino acid sequence of the corresponding surface protein and to examine the properties of the gene that codes for that protein.

The sequences of several cDNAs for different trypanosome surface proteins have now been determined and the corresponding amino acids sequences of the proteins deduced. This work has shown that the different surface proteins have similar amino acid sequences near the C-terminal ends and very different amino acid sequences through the rest of the molecules. We now know that the C-terminal portion is always attached to the trypanosome's outer membrane and that the variable portion of the protein is directly exposed to antibodies of the the immune system. Thus, when there is a switch from one surface protein to another, a new variable portion is presented to the immune system and a new set of antibodies must be raised.

The cloned cDNA molecules for the surface proteins can also be used as probes in a biochemical technique called nucleic acid hybridization to examine the locations of the corresponding genes in trypanosome chromosomes. These experiments have revealed that the trypanosome chromosomal DNA contains as many as 1000 genes for different surface proteins. Furthermore, one, and only one, of these 1000 different genes is expressed at a time. Before such a gene can be expressed, it undergoes a duplication and the duplicated copy is inserted into a special place in one of the chromosomes called an "expression site." In this expression site the duplicated gene is transcribed into RNA and the RNA is translated into the surface protein. When a switch occurs, the duplicated gene copy in the expression site is removed and replaced by the duplicated copy of a second gene. The displaced first copy disappears, apparently because it is degraded after leaving the expression site. The new gene copy in the expression site is then transcribed and translated into the second

surface protein. This spontaneous switching process seems to be able to continue almost indefinitely, each time generating a new surface protein against which the immune system must mount a new immune response.

This molecular mechanism on the genetic level can be thought of as rather similar to the use of a tape recorder or a cassette player. It is as if one has a collection of 1000 different tapes and only one tape can be played in the cassette player at a time. Furthermore, in order to be played, the tape must be first copied and the copied version inserted into the player. After that copied version has been played, it is discarded and a duplicate copy of a second tape in the collection is then inserted into the player, and so on. In that fashion each original tape in the collection is left untouched and the copied versions are the ones that are actually played. This "cassette model" analogy can be carried a little further. Sometimes a copy of the front half of one gene and a copy of the back half of a second gene are simultaneously inserted into the "cassette player" to create a new hybrid gene for which there is no identical version in the original collection in the 1000 genes. Thus, the duplication event provides a mechanism for creating new genes in the expression site that give rise to new surface proteins. In other words, by having the capacity to mix and match segments of genes during the duplication and insertion process, the trypanosome can potentially generate a virtually infinite number of surface proteins from a finite number of surface protein genes.

The switch from one surface protein to another occurs spontaneously at a rate of about one switch per million trypanosomes per doubling time. This is about the switch frequency necessary for the trypanosome population as a whole to keep one step ahead of the immune system. This strategy for keeping ahead of the immune system is so ingenious, especially considering that it permits the successful creation of new surface protein genes, that it seems unlikely a vaccine against African trypanosomes will be developed in the near future. This does not mean that the situation is hopeless. For example, we still do not understand what are the molecular mechanisms that trigger the actual transcription of the duplicated gene once it has arrived in an expression site. It does mean, however, that much more research is necessary before we completely understand the entire switching process at the molecular level.

Immune Evasion by the Malaria Parasite

I would now like to turn to a discussion of malaria. This disease is caused by a very different parasite that belongs to the genus *Plasmodium*. These organisms are transmitted to humans or animals during the bite of the *Anopheles* mosquito. Malaria occurs throughout tropical regions of Asia, Africa and Latin America where it has resisted all attempts to eradicate it. It is possible to eliminate malaria, however, if the conditions are right. For example, malaria used to occur here in Iowa and throughout the Mississippi River valley. Laura Ingalls Wilder, who wrote *The Little House on the Prairie*, talks about malaria in South Dakota during the 1800's in one of her other books. However, transmission of malaria has been eliminated from this country, mainly by draining most of the swamps and extensively spraying the *Anopheles* mosquitoes with DDT. In the endemic areas of today, and especially in the rain forest areas, it is not ecologically feasible to use a similar eradication plan.

The life cycle of the malaria parasite is more complicated than that of the African trypanosome. When an infected mosquito bites a person, the *sporozoite* stage of the parasite enters the bloodstream. The sporozoites stay in the bloodstream for only a few minutes. As soon as the blood containing them passes through the liver, they leave the blood and go inside the liver cells. Once inside the liver cells they are hidden from the immune system. They remain in the liver for about two weeks, after which they burst out into the bloodstream again. This developmental stage of the parasite is called the *merozoite* stage. The merozoites also remain free in the blood for only a few minutes. They quickly enter red blood cells and are again hidden from the attack of the immune system. Within the red blood cells they grow and multiply, eventually killing the cells to spill out and invade still more red blood cells. A few of the newly formed parasites are *gametocytes*, which can be taken up by mosquitoes to complete the life cycle.

Thus, the strategy used by malaria parasites to evade the immune system is to spend most of the time hiding within either the liver cells or the red blood cells. However, it turns out, as mentioned earlier, that adults who have survived repeated attacks of malaria during childhood often possess considerable resistance to further attacks. This resistance has been shown to be immune-mediated. It must be periodically "boosted" by infection with new sporozoite parasites from the mosquitoes. For example, adults who have acquired this

immunity and leave a malaria-endemic area for several years run the risk of new malaria attacks when they return if they are bitten by infected mosquitoes. Nevertheless, the existence of this naturally acquired immunity provides hope that it will be possible to develop a vaccination against malaria and is the basis for substantial research activity in this area.

In recent years the search for a possible malaria vaccine has focused on the use of recombinant DNA techniques to clone specific malaria genes, just as the study of trypanosome surface proteins has relied on examinations of their cloned genes. The approach has been to collect in the laboratory large numbers of the specific developmental stages of the malaria parasite and to construct cDNA libraries of the mRNAs isolated from these different stages. The stage-specific cDNA libraries are then used to identify and characterize the genes for malaria proteins that interact with the immune system.

The first malaria protein whose gene was cloned and characterized is the major protein on the surface of the sporozoite stage, i.e., the developmental stage of the parasite transmitted during the bite of the mosquito. It turns out that this stage of the malaria parasite is covered by many identical copies of a single surface protein, just as are the bloodstream forms of the African trypanosome. However, this malarial surface protein, called the *circumsporozoite* protein, is much different than the trypanosome surface proteins. As first shown by the research of Victor and Ruth Nussensweig, and their colleagues at New York University, we now know that about half of the circumsporozoite protein—the middle half—is comprised of tandem repeats of just a few amino acids. In *Plasmodium falciparium*, the species that causes the most serious form of malaria in humans, the circumsporozoite protein has 41 repeats of the simple sequence,— asparagine-alanine-asparagine-proline—$(\text{Asn-Ala-Asn-Pro})_{41}$. It is not clear why this surface protein has these amino acid repeats but their presence is so unusual that we believe they play an unknown role in helping the malaria parasite avoid the immune response.

Remarkably, when cDNA libraries were constructed of the merozoite and red blood cell stages of the malaria parasite, these developmental stages were also found to possess many proteins that contain short amino acid repeats. In fact, relatively few malaria proteins have been studied which do NOT have these tandemly repeated amino acids. The number, sequence and length of these repeats vary with the

different proteins, but the overall phenomenon of internal repetitiveness seems to be very common in malaria proteins. Thus, they must have some importance for the life cycle of the parasite and/or the interaction of the parasite with the immune defenses of its mammalian hosts.

One hypothesis about the repetitive malaria proteins advanced by several laboratories, including our own, is that they serve to divert the immune response of the host from more vulnerable regions of the parasite surface. This "smokescreen" hypothesis postulates that the tandem repeats directly or indirectly cause a hyperstimulation of non-protective antibodies leading to a less effective "protective" response that would kill the parasite. This suggestion is supported by the finding that when experimental animals are injected with synthetic versions of these amino acid repeats, antibodies are raised against the repeats, but these antibodies do not protect the animals from getting malaria. Yet, if the animal is "vaccinated" with X-irradiated sporozoites (which cannot divide), protection against malaria does occur. Thus, it may be that the presence of so many small repeat sequences fools the immune system into attacking the wrong things, and it is only with time and repeated infections that a protective immune response against crucial regions of the parasite begins to build up. This reasoning would explain why children are more susceptible to malaria than are adults who have had repeated malaria infections during their youth. One of the major challenges now in malaria research is to identify the proteins or other antigens that trigger this "protective" immune response which is so slow to materialize. These antigens will be the ones on which a possible vaccine must be based.

River Blindness—A Disease Caused by a Worm

River blindness, or onchocerciasis as it is formally called, is caused by still another parasite that is spread by small black flies living near the streams and rivers in central Africa and parts of Latin America. The flies harbor a larvae form of a worm called *Onchocerca volvulus.* When the flies bite a person, these larvae enter the body and eventually mature into adult worms—a process that can take a year or more. The adult female worms are encapsulated within nodules of cartilage and collagen that can often be noticed as lumps just under the skin of an infected person. A female worm can grow to more than 12 inches in length and live for as long as 10 years in its nodule. It produces

microfilariae, or baby worms, at a rate of 1000 or more per day. These microfilariae move throughout the skin and can be picked up by the black flies to complete the parasite's life cycle. Unfortunately, in a heavily infected person the microfilariae also enter the eyes and cause lesions. In severe cases, these lesions result in complete blindness—hence, the name river blindness. About 50 million people in Africa are at risk of catching this disease and about 12 million people are estimated to be infected. In some remote villages of sub-Saharan Africa, most of the adults over the age of 40 are either blind or suffering eye problems. This severely limits the productivity and economic independence of the people living in these villages. Fortunately, a promising new drug called ivermectin that has been available for only about 2 years effectively kills the microfilariae without serious side effects. This drug does not kill the adult worms, however, and must be continually taken to suppress the production of microfilariae. Thus, like drugs that act on the malaria parasite, ivermectin can be used to control the parasite in individuals, but it does not eliminate the disease. Other means of combating the disease must be found before it can be eradicated.

Onchocerca volvulus infects only humans and chimpanzees. It does not survive when injected into the conventional laboratory animals such as mice, rabbits, guinea pigs, etc. Thus, river blindness is very difficult to study in the laboratory—there is no available "animal model" for the disease, as we say. Likewise, the black flies that transmit the parasite are very difficult to rear in the laboratory which complicates the study of the parasite itself. In recognition of these obstacles to investigating the disease, we decided several years ago to use recombinant DNA techniques as tools for learning more about the development of *Onchocerca volvulus* and its interaction with the immune system. For example, nothing is known about how this parasite avoids destruction by the host immune response. Similarly, very little is known about the events that accompany the maturation of the microscopic larvae into the 12-inch-long adult female.

To begin our research we needed to obtain the purified parasites. An official of the World Health Organization, Brian Duke, was instrumental in obtaining permission from health officials in Guatemala in Latin America and in Mali and Cameroon in Africa for us to surgically remove nodules from river blindness patients. The nodules were routinely removed under local anesthesia from more than 100 river blindness patients and the adult worms were isolated from these

nodules. RNA and DNA were purified from the worms by standard biochemical techniques and used to construct recombinant DNA libraries. The libraries were constructed in such a way that harmless laboratory bacteria containing the cloned parasite genes are capable of synthesizing specific parasite proteins. Thus, the bacteria become factories for producing proteins that are normally found in or on the parasite itself. This eliminates the need for large numbers of parasites to obtain these proteins, solving some of the problems that arise because there is no "animal model." Furthermore, we have been able to identify parasite proteins made by the recombinant bacteria that are recognized by antibodies in the serum of river blindness patients. Some of these proteins have become the focus of our studies because they are proteins that may be involved in the mechanisms that the parasites use to escape elimination by the immune system.

During these studies we have discovered a curious feature of RNA synthesis in *Onchocerca volvulus*. Normally, the mature mRNA molecules of a cell are derived entirely from the corresponding gene in the DNA of the nucleus. In *Onchocerca volvulus* and in other nematode organisms, some of the mRNAs contain a small stretch of RNA (22 nucleotides) at one end that does not come from the corresponding gene encoding the protein. This small region of RNA is derived, instead, from another gene that is far removed from the protein-coding gene. The initial RNA molecules synthesized from these two genes are apparently *trans*-spliced, or joined together, to yield a mRNA that is a composite of both of the initial RNAs. It is not clear why this *trans*-splicing occurs in these organisms but there is no evidence for its presence in mammalian cells. Thus, one possible attack on the parasite would be to identify a drug that interferes with RNA *trans*-splicing but is harmless to RNA synthesis and processing in the mammalian cells. Although no solutions for eradicating river blindness are available yet, the new methods for studying the parasite that we and other laboratories are now using should lead to a better understanding of how this parasite matures in the body and how it avoids the immune response.

Art, The University of Iowa, and Tropical Diseases

Finally, let us finish today by leaving the world of molecular biology and entering another arena related to medicine—art. In tropical areas of the world, artists are profoundly influenced by their daily confrontation with infectious disease. Many examples of this confrontation

are found in the collection of African art here in the University of Iowa Museum. The Songye people of Zaire, for example, produce wooden statues or figures which are given magical powers by the accumulation of materials applied by the herbalist/magician/healer of a village. These figures often have facial features that are aggressive and threatening, and are intended to protect the people of a community from the threat of diseases. This first figure possesses a head and body covered with the scars and pustules of a smallpox victim and may have been created during one of the many smallpox epidemics in central Africa during the last century.

This second figure is from the Kongo people, also of Zaire, and is one of the most dramatic figures in the African art collection here at Iowa. It is a symbol of the pain, suffering and agony inflected on the people by disease. This "nail fetish" symbol would be in the possession of the village healer, and relatives and/or friends would drive nails or sharp objects into it in an effort to drive sickness out of an ill person. An equivalent action in our society would be to light candles at a church on behalf of a sick or bereaved person.

The third, and last, figure is also from the Kongo people of Zaire. It has a feather headdress and leopard's teeth draped over its shoulders. This object is, again, intended to protect a family from diseases when additional materials are added to it by clients of the village healer. This figure is different from the other two figures, though, in one important feature. Clenched in its teeth is a medicinal plant or twig. Over the years we have learned that many of the so-called "magical" cures used in tribal cultures around the world are not so magical after all. Quinine was first found in the bark of a tree in Peru. One of the most promising "new" anti-malarial drugs comes from a little-known plant in China that was first described as having anti-malarial activity by the Chinese in 1596. Ivermectin, which has become a miracle drug against parasitic worms, was discovered in a soil bacterium first isolated in Japan.

We stand today at the doorstep of some tremendous advances in tropical disease medicine that I think will take place during the remainder of this century and into the 21st century. By combining the ancient understanding of these diseases with new high-tech approaches such as molecular biology techniques, we have a chance to conquer these tropical diseases, just as smallpox has been conquered, thereby providing a higher quality of life to millions of people in the world.
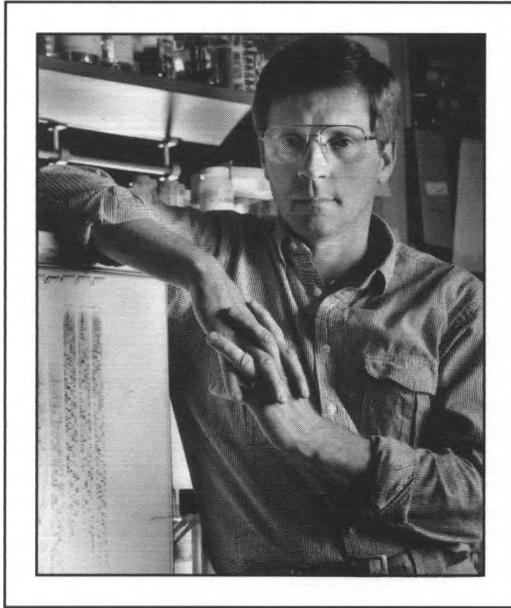
John E. Donelson joined the Department of Biochemistry at the University of Iowa in 1974 and was appointed an Investigator of the Howard Hughes Medical Institute in 1989. His research interests in tropical diseases date back to 1980 when he was a visiting Fogarty International Scholar in Kenya. His more than 100 scientific publications, including a Scientific American *summary article, describe studies on sleeping sickness, river blindness, malaria, Chagas' disease, leishmaniasis and schistosomiasis. He is a recipient of the Burroughs-Wellcome Molecular Parasitology Award and co-director of a summer course on "Biology of Parasitism" at the Marine Biological Laboratory in Woods Hole, Massachusetts.*