Scrutinizing Sleeping Sickness

Molecular biologists are thronging to the study of sleeping sickness, fascinated by the novel genetic mechanisms used by the parasites that cause the disease

In Africa, along the equator, live large brown flies with long, thin snouts whose painful bites cause red welts. These are the tsetse—the dread flies that carry the disease known as African trypanosomiasis, or sleeping sickness.

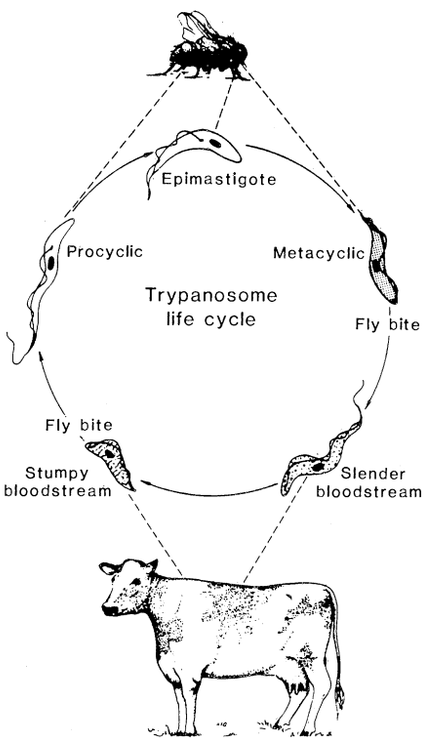
Sleeping sickness is an ancient disease of both humans and animals. In the past, it has decimated populations. Even today, it affects about 1 million people in central and east Africa and 20,000 new cases appear each year. The disease also has a tremendous social and economic impact because it prevents livestock from living in an area of Africa that is larger than the continental United States. And it is a disease that defies vaccination because the trypanosomes that cause it have developed immensely clever ways to foil the immune system.

Parasites have evolved many different ways of surviving in their mammalian hosts by evading the immune response, but the system displayed by trypanosomes must be counted as one of the most successful. Trypanosomes are able to switch their surface coats at regular intervals, so that every time the host develops antibodies to the organisms, the antibodies become ineffective. In this way, the trypanosomes always remain one step ahead of the host's immune defenses. The means by which this antigenic variation is achieved is now under intense scrutiny by a growing cadre of molecular biologists who hope that their work may one day lead to new ways to control sleeping sickness. In any event, the work is giving them new insight into gene regulation and is revealing that trypanosomes are fascinating in their own right.

If the number of investigators working in a field is any indication of its importance, the study of African trypanosomes must be ranked high. The field, says John Boothroyd of Stanford University, "is expanding very rapidly—much like [the study of] *Escherichia coli* did." "Most discoveries now are being made in three labs simultaneously," says George Cross of Rockefeller University." "It's such a competitive field it's just impossible," sighs Nina Agabian of the University of California at Berkeley.

The molecular biologists come to the study of trypanosomiasis against a background of a century of work on the disease. The studies were begun by Europeans who encountered sleeping sickness when they began to colonize Africa and were terrified that it might spread. Sleeping sickness, says Boothroyd, was "the AIDS of the turn of the century." In fact, he remarks, "This disease made AIDS look like nothing. Two-thirds of the people on the north shore of Lake Victoria died and no one knew what was causing it. All the big colonial powers were there and they were worried that the disease would disseminate to enormous numbers of people."

The disease, by all accounts, is a horrible one. It begins insidiously with malaise, lassitude, and a low-grade fever. Then the person develops fevers and severe sweating, especially at night. His face gets puffy and he becomes drowsy in the daytime and an insomniac at night. Finally, the person becomes so sleepy that he no longer eats. He suffers seizures, mental deterioration, and tremors. Eventually he becomes comatose and dies.



Life cycle of African trypanosomes

The trypanosomes enter the host's body when he is bitten by an infected fly. There they multiply and can be taken up by another tsetse fly to infect another host. [*Courtesy* Immunology Today]

Europeans who traveled to Africa and saw firsthand the ravages of trypanosomiasis were invariably shaken. Sir Winston Churchill, for example, visited east Africa in 1907 and arrived in Uganda. At first he was charmed. Uganda, he wrote, is "a fairy tale. You climb up a railway instead of a beanstalk, and at the end there is a wonderful new world." Later, when he saw the epidemic of sleeping sickness there, he changed his imagery. Uganda, he said, is "a beautiful garden of death."

The mystery of trypanosomes was how they manage to survive in their host. These protozoa—single-celled organisms—enter a person's bloodstream when he is bitten by an infected tsetse fly and multiply there—exposed at all times to the person's immune system. Only at the end of the infection do the trypanosomes invade the central nervous system. Ordinarily, in such circumstances, an organism would be wiped out by the immune system. But trypanosomes survive and flourish, reaching enormous concentrations in the bloodstream. In laboratory rodents, says Paul Englund of Johns Hopkins University School of Medicine, there can be nearly as many trypanosomes as red blood cells before, as Englund says, "the rat keels over and dies." Humans and animals that develop natural infections probably have fewer trypanosomes in their blood, according to Englund, but they face the additional complication that the organisms eventually infect the central nervous system.

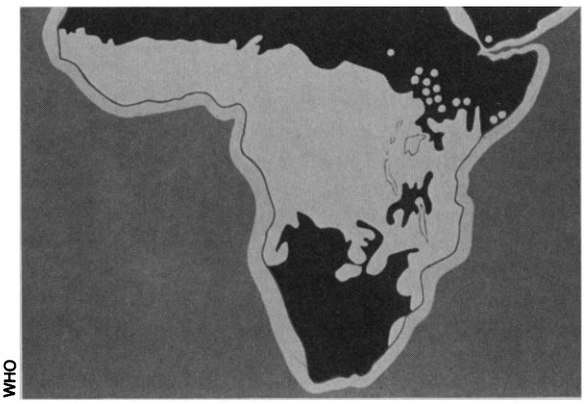
Researchers from several laboratories over the past decade have learned the nature of the trypanosome's tricks. What the organism does is to constantly vary its protein surface. Typically, a fly will inject trypanosomes into the host's bloodstream and the host will make an enormous number of antibodies—enough to destroy at least 99 percent of the trypanosomes within a week or so. But the few remaining trypanosomes will have changed their coats, eluding the host's immune system and multiplying with impunity until, again, the host manages to make antibodies to destroy 99 percent of the organisms. Once more, the remaining trypanosomes will have changed their coats. This process continues until, finally, the organisms invade the host's central nervous system and the host dies.

Trypanosomes change their coats by turning off one gene coding for a coat protein and turning on another. The entire surface of a trypanosome is covered by about 10 million molecules of a single protein, called a variable surface glycoprotein, or VSG, and so it is only necessary to turn on a single new gene to completely change the surface coat. The trypanosomes are thought to have anywhere from several hundred to several thousand VSG genes which are classified into two groups based on partial homologies in the carboxyl termini of the proteins they code for. Other than that, the individual genes have unrelated sequences. The genes are average or a bit below average in size—about 1500 nucleotides in length.

Among these VSG genes are about 12 to 14 that are always expressed first when an infected fly bites a mammalian host. These genes, called metacyclic, look just like the other VSG genes and their antigens appear on the organisms all at once—one trypanosome will have one metacyclic gene expressed, one will have another, and so on. After about 5 days, all the metacyclic genes are turned off, in a matter of a few hours, and the infection continues with the expression of one VSG gene after another.

Molecular biologists have reached no consensus on whether the VSG genes are expressed according to any sort of pattern. Some say that there is a pattern, although not a rigidly determined one. Lex Van der Ploeg of Columbia University describes it as "a sloppy order," meaning, he says, that the order is not completely random but neither is it predictable. Others say they see no order at all. John Donelson of the University of Iowa suggests that these differences of opinion may stem from differences among the various strains of trypanosomes. There is one intriguing hint, however, that the expression of VSG genes may obey some sort of rules, at least initially. Investigators find that the VSG gene that is being expressed when a tsetse fly bites an infected person is the first to appear after the metacyclic genes when the tsetse reinfects someone else.

The phenomenal antigenic variability of trypanosomes has captured the attention of molecular biologists interested in the control of gene expression. The question that immediately came to mind was, How can an organism that has only two to three times as much DNA as a simple yeast cell have genes for so many surface proteins and yet express only one at a time? The answer seems to involve some intriguing mechanisms, never before seen in any organism but possibly not limited to trypanosomes.



Africa at risk

Like a belt across the continent (light area), the area where the tsetse live is nearly devoid of domestic animals. People living in this belt are at constant risk for trypanosomiasis, sleeping sickness.

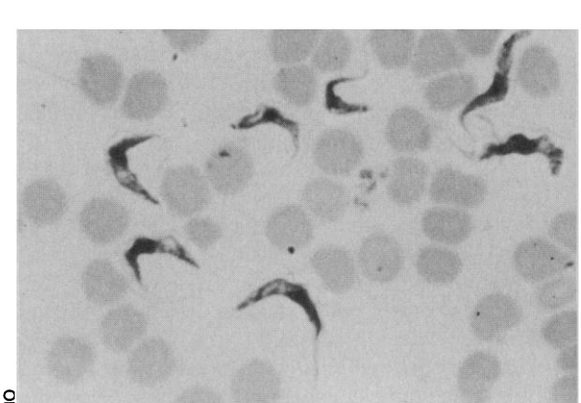
The key feature of these VSG genes is that they are never expressed unless they are at the ends of chromosomes. But most of these genes are located in the interior of the chromosomes, which means that to be expressed they must be moved. Piet Borst of the University of Amsterdam in the Netherlands and Cross of Rockefeller discovered that trypanosomes duplicate a gene to be transcribed and that this duplicate appears in an "expression site" at the end of a chromosome. There it replaces another surface antigen gene which, Borst says, "disappears—it is probably destroyed." Borst and Van der Ploeg find that there are at least three and possibly several more expression sites among the 200 ends of the 100 or so chromosomes of the trypanosomes.

The investigators call this gene expression system a cassette model, meaning it is like a cassette tape recorder. To play a tape, you first take out the one there and then insert a new one in the slot. But what, if anything, determines which chromosome ends are expression sites for VSG genes?

The trypanosome chromosomes range in size from about 50,000 base pairs of DNA—the size of the DNA of a small bacterial virus—to 2 million base pairs. Unlike human chromosomes or chromosomes of other higher organisms, the trypanosome chromosomes are too small to see in the light microscope. For that reason, it is impossible to determine, by changes in a chromosome's size, that it has recently gained or lost a gene. But Charles Cantor and David Schwartz of Columbia University have recently developed a system for separating large DNA molecules by making them reorient in perpendicular electric fields, and this method also allows these investigators to do cytogenetic studies of these organisms. Using this system, Van der Ploeg, Borst, Schwartz, and Cantor were able to determine that one of the expression sites is on a large 2-million-base-pair chromosome, one is on a large chromosome of undetermined size, and a third is on a 550,000-base-pair chromosome.

Although most of the VSG genes seem to be in the interiors of chromosomes and to be moved to expression sites before they are active, some are normally located at the ends of chromosomes. It is still uncertain whether these genes are activated where they are or are moved to expression sites where they are turned on.

No one knows what determines which gene will be expressed when nor which expression site will be used. But one hint that gives researchers direction in their search for the gene switching rules is a



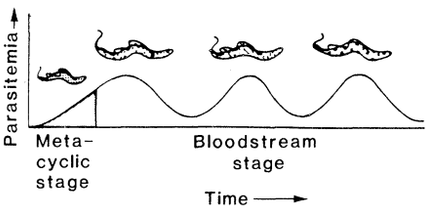
Trypanosomes fill the blood of infected hosts

In constant motion, the trypanosomes dart about in the blood, looking like "tiny snakes," according to Scottish physician David Bruce, who was among the first to connect the parasites to sleeping sickness.

recent observation by Borst and his associate André Bernards that unexpressed VSG genes at the ends of chromosomes are modified in some way. These include genes that seem to be normally at the ends of chromosomes as well as genes that are in expression sites but that are not turned on. The nature of this modification is unknown, and the only reason Borst and Bernards know it exists at all is because it renders the DNA of the genes inaccessible to restriction enzymes that are normally able to cut it.

Still another oddity of gene expression in trypanosomes is the discovery by Cross, Borst, and Boothroyd that VSG messenger RNA's (mRNA) have a small 35-nucleotide sequence spliced to them. It is always the same sequence and it is always at the beginning—the 5′ end—of the mRNA's. At first, the investigators were hopeful that this 35-nucleotide sequence would lead them to the mechanism for switching on VSG genes. But it seems to be completely nonspecific. A number of research groups, including those that initially discovered this "discontinuous mRNA synthesis" and also including groups headed by Agabian, Donelson, and Frank Richards of Yale, find that most and possibly every mRNA of the trypanosomes—including mRNA's that have nothing to do with the surface antigen genes—has the 35-nucleotide sequence spliced onto it. "We have yet to find a messenger that doesn't have it," Agabian says.

Borst and his colleague Titia VeLange and, independently, Agabian and her colleagues recently looked to see whether the 35-nucleotide sequence appears on the mRNA's of other trypanosomes. So far, they find it in South American trypanosomes, which cause Chagas' disease, a chronic disease of the heart and other muscle tissue, and in the related leishmania parasites, which cause a disease that, like leprosy, produces skin ulcers and disfigurement. Agabian and Borst are now looking for the sequence in other organisms.



Antigenic variation in an infection

Every time the infected host makes antibodies to wipe out nearly all of the trypanosomes in his blood, the remaining trypanosomes will have changed their coats, allowing them to elude the immune system. [*Courtesy* Immunology Today]

As yet, no one knows how or when the 35-nucleotide sequence is added to mRNA's. It is known that the sequence is transcribed from repeated DNA sequences that are 1350 base pairs in length and, as Boothroyd and Borst recently independently showed, that the 35-nucleotide mRNA segment is initially derived from a short 137-nucleotide sequence. Beyond that, the segment is a mystery.

Since all of these studies of gene expression in trypanosomes are at the level of description, it is very difficult to make sense of them. The central question—how is gene switching regulated?—is unanswered. "We're all lacking that marvelous new lead that we need to pin this thing down," Cross says.

At the same time as molecular biologists are attempting to understand the mechanisms of gene switching in trypanosomes, investigators are looking closely at the nature of the surface proteins of these organisms. One major question is how the structure of the proteins may make them antigenically so diverse. The point here is that in order to evade the immune system so successfully, each surface protein must have little or no immunological cross-reactivity with previously exposed antigens. And yet the surface antigens, like any other proteins, must have, by chance, some regions that resemble each other in structure. As Richards explains, the person infected with trypanosomes makes such an enormous number of antibodies to them that any cross-reactivity at all between the surface antigens exposed at different times would be expected to render the immune attack successful.

This anomaly intrigued Richards and his colleague Thomas Lalor. "It began to interest us why there is no cross-reactivity," Richards says. They examined the potential secondary structures of five full length and five partial amino acid sequences of different VSG proteins and determined that the proteins may be folding in quite different ways, exposing only small sections—and different sections—of their structure each time. The lack of any apparent immunological cross-reactivity, Richards speculates, in part seems to have to do with how the proteins are organized. "They are like coils and only a small cap on their sur-

The AIDS Connection

Sleeping sickness is caused by African trypanosomes, which are protozoa. AIDS seems to be caused by a type of RNA virus. At first glance, the two diseases seem to have little in common. But several groups of investigators are currently looking to see if the trypanosomes may have similar effects to those of the AIDS viruses on the immune system and, if so, whether that can lead to new insights into either disease.

John Mansfield of the University of Louisville, for example, finds that trypanosomes produce immune system changes in mice that very much resemble the changes seen with AIDS. Whether the same effects occur in humans is unclear, but a team of scientists from the Centers for Disease Control, the National Institutes of Health, and Zaire are currently in Zaire testing and comparing patients with AIDS to patients with any of several parasitic diseases, including sleeping sickness, to see how these parasites affect immune functions. Results should be available in December, according to Thomas Quinn of Johns Hopkins University School of Medicine and the NIH.

The investigators in Zaire are not just looking to see if sleeping sickness and AIDS produce the same immune suppression. They are also investigating a hypothesis that AIDS is prevalent in Zaire because trypanosomiasis and other parasitic infections that occur in Zaire render patients less able to fight off the AIDS virus.

A final AIDS connection is with suramin, a drug that is commonly used to treat sleeping sickness. Recently, Robert Gallo and his associates at the NIH discovered that it is effective against the putative AIDS virus in vitro (*Science*, 12 October, p. 172). They are now beginning studies to see if it can be administered to AIDS patients. Samuel Broder, a member of the NIH group, attributes the drug's effectiveness against both African trypanosomes and AIDS viruses to serendipity. But it is another intriguing link between the ancient disease of trypanosomiasis and the recently discovered AIDS.—**G.K.**

face is exposed," he says. For that reason, two different surface proteins may have virtually no similarities in the sections of their structure that are exposed to the host's immune system.

As intriguing as the biology of the trypanosomes is, researchers are still very much aware that the organisms cause a serious disease and that, eventually, their work may lead to some way to prevent or treat it. Most feel that a vaccine for trypanosomiasis is unlikely. There is no way to vaccinate against the hundreds or thousands of surface coats that these organisms rely on for their disguise. The only possibility—and it is a slim one—is to vaccinate against the 12 to 14 different coats that appear at the start of an infection. However, as Richards points out, "If we suppress these, it is entirely possible that others would appear." Moreover, there are numerous strains of trypanosomes, each with slightly different antigen collections. Being realistic, Cross says, "The outlook for a vaccine is about as pessimistic as it can be."

Of course, there is always the possibility that a drug could be developed to interfere with some vital process that is unique to trypanosomes. The few drugs used to treat sleeping sickness are very toxic and have serious side effects, so new and safer drugs would be welcome. And trypanosomes certainly do seem to be unusual. According to Cross, "Almost every metabolic pathway looked at in trypanosomes is substantially different [from what is seen in other organisms]. I'm sure there is a wide range of pathways amenable to chemotherapy." Nonetheless, according to Cross, "There is almost no work on drug development going on. No major drug company that I'm aware of is screening drugs for use against trypanosomes." The reason is that such drugs would not be profitable—the people living in trypanosome-infested countries are frequently desperately poor.

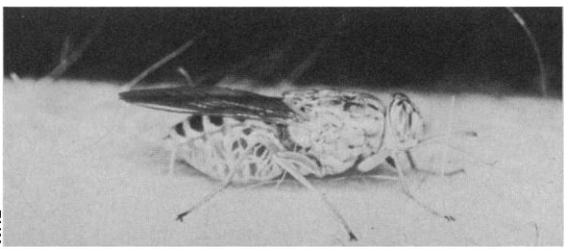
Of course, there is always the chance that molecular biologists may stumble on a new treatment for sleeping sickness as they come to understand, say, VSG gene switching or discontinuous mRNA synthesis. It would be a rational drug design as opposed to drug screening. But Boothroyd, for one, is pessimistic that this approach will lead to anything soon. "It's a question of whether the chemistry is up to it," he remarks. "It is a quantum leap to go from [an understanding of a unique pathway] to having a drug in hand."

But there are other reasons for studying trypanosomes. Many of the investigators who are immersed in the technicalities and phenomenology of trypanosome gene expression see a broader aspect to their work. Cantor, for example, hopes that an understanding of trypanosome molecular biology may lead to insight into "what does it mean to be a chromosome?" In other words, what determines which genes will be arranged together on a chromosome? He notes that "organisms like trypanosomes that switch [the genes of their] chromosomes around at a frightening pace operate under different rules." The hope is that the trypanosome rules may lead to rules for other organisms whose chromosomes are more quiescent. As Cantor notes, the very stability of most other chromosomes makes them difficult to get at. "If the rule is that nothing happens, how can you understand what the rule is?" he asks.

Borst also feels that studies of the peculiarities of trypanosomes may lead to an understanding of other organisms. For example, he finds that trypanosomes add 6 to 8 base pairs to DNA to the ends of their chromosomes with each cell division and then, after the chromosomes have grown by thousands of base pairs, they delete large chunks from the ends. This growth and retraction of chromosome ends is thought to occur in other organisms as well, although not in so flamboyant a manner. Because they "do everything in excess," trypanosomes offer a way to study such phenomena, Borst says.

Cross, on the other hand, wonders whether trypanosomes really are like other organisms. They are very ancient on the evolutionary scale, he notes, and, based on an analysis of their cytochrome c, are farther removed from yeast than yeasts are from humans. For that reason, he says, they "are likely to be different" from mammals in their genetics and biochemistry and he studies them because they are interesting in their own right.

Boothroyd says that the study of trypanosomes has stimulated him and others to look at other parasites. In the trypanosome studies, he says, "so many interesting things are coming out that really have no precedent—that's what makes it so exciting. Now there is an enormous stimulation of interest in parasitology in general. Much of that is a direct spin-off of the work on trypanosomes."—**GINA KOLATA**



WHO

A tsetse fly feeds

In starting the trypanosomiasis infection cycle in human hosts, the tsetse fly acts as a "filter" for the pattern of variable surface glycoproteins that follows.

Additional Reading

1. John J. McKelvey, Jr., *Man Against Tsetse* (Cornell Univ. Press, Ithaca, N.Y., 1973).
2. W. J. Murray *et al.*, *J. Protozool.*, **31**, 65 (1984).
3. J. Boothroyd, *Annu. Rev. Microbiol.*, in press.
4. M. Pearson, R. J. Nelson, N. Agabian, *Immunol. Today* **5** (No. 2), 43 (1984).

WHO

Deserted village in West Africa

One effect of sleeping sickness is to decimate local populations; the few survivors frequently move to safer areas.