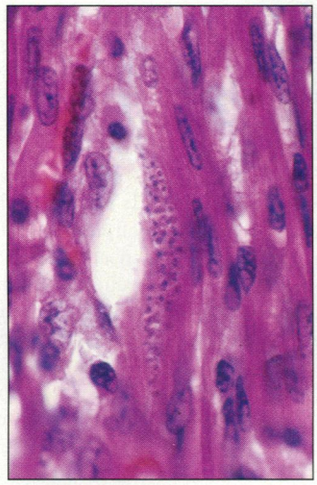
BRAZIL

A Deadly Parasite Spurs Up-To-the-Minute Biology

CAXAMBU, BRAZIL—When hundreds of biologists from Brazil and elsewhere gathered here for a recent meeting, the work they discussed would not have been out of place at Cold Spring Harbor or Heidelberg: genome mapping and sequencing, protein crystallography, and rational drug design. But all this up-to-the-minute science was aimed at a traditional focus of Brazilian biology: *Trypanosoma cruzi*, the insect-borne parasite that causes Chagas disease. Discovered 86 years ago by a Brazilian doctor named Carlos Chagas, the parasite sparked a public health campaign and a national research effort. *T. cruzi* is now studied by Brazilian biologists of every rank, from the greenest undergraduates to the most senior laboratory chiefs, and it is the driving force for international collaborations and the first major genome project organized and led by Latin Americans.



ARMED FORCES INSTITUTE OF PATHOLOGY

MARTIN DOHRN/PHOTO RESEARCHERS

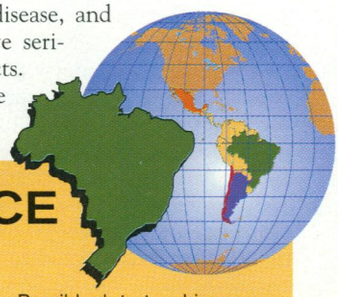
Nasty bite. A bloodsucking triatomine bug transmits the protozoan *T. cruzi*. Infected heart muscle *(left)* reveals a cluster of the organisms, to the right of the space.

One reason for the organism's broad influence on biology in Brazil is that it has proved a tenacious foe. Today, after eight decades, researchers say they are more confident than ever that they are getting the upper hand in their battle with *T. cruzi*. But success is not yet in their grasp: Each year in Brazil, several thousand people die of heart and digestive problems brought on by the disease, which infects as many as 18 million people worldwide. So far, researchers have not been able to develop a cure or an effective treatment. Nor have they got a cheap, specific blood test. As a result, the battle against *T. cruzi* remains a driving force in Brazilian biology, propelling its first ventures into the front lines of molecular biology.

The battle began when Chagas, at age 29, went to fight malaria among railroad workers in a rural town north of Rio de Janeiro. While there, he noticed unusual symptoms among the local people—lethargy, shortness of breath, irregular heartbeat—which he guessed were caused by an unknown parasite. He quickly tracked down the vector, a long-snouted triatomine bug that lived in thatched houses and sucked blood from the inhabitants as they slept. From the bug, Chagas extracted a whiplike protozoan, kin to the trypanosome that causes sleeping sickness in Africa (*T. brucei*). Chagas proved that *T. cruzi* could infect monkeys; then he isolated it from a cat in a bug-infested house; and finally he isolated it from the blood of a girl who had symptoms that are now recognized as classic signs of acute infection.

Chagas accomplished all that in 1909. But the war against *T. cruzi* got off to a slow start, in part because the parasite is elusive. After an acute infection that lasts several weeks, the population of organisms in the bloodstream declines sharply, making it hard to isolate, and about 10% to 20% of people who are infected never exhibit symptoms. As a result, Chagas spent the years before his death in 1934 trying to persuade scientists that he had found a real public-health hazard.

It was not until the 1970s that the Brazilian government and the World Health Organization (WHO) joined forces to develop a better scientific understanding of the disease. They have funded research on improved blood tests, isolated many strains of *T. cruzi*, instituted blood-bank controls to stem the transmission of the disease by blood donors, and supported research on the parasite's biology. All this has been done in the hope—so far unfulfilled—of finding an effective way to prevent the parasite from invading human cells or reproducing within them. The two standard drugs—nifurtimox and benznidazole—are not effective in treating chronic disease, and they can have serious side effects. Vaccines have been ruled



BRAZIL AT A GLANCE

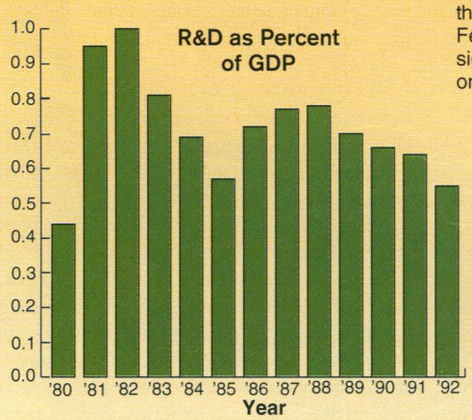
The Big Picture

Population, 1994:	158.7 million
GDP, 1993*:	$428 billion
Total R&D Spending, 1992*:	$2.385 billion
Scientists and Engineers, 1990:	65,000

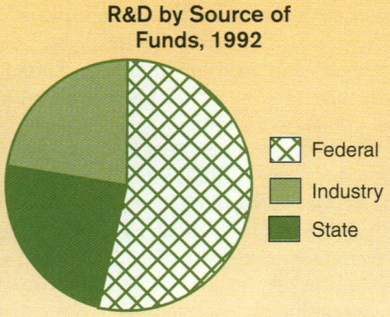
* In 1993 dollars.

SOURCES: GDP, R&D: MCT; Scientists and Engineers: Latin American Academy of Sciences; Population: 1994 CIA World Fact Book

Researchers in Brazil look to two big agencies for R&D funding: the Conselho Nacional de Desenvolvimento Científico e Tecnológico for stipends and equipment grants, and the Financiadora de Estudos e Projetos for technology-development loans. Agricultural and space research get support from other agencies, and the government funds 10 special institutes under the Ministry of Science and Technology (MCT). Overall R&D spending is increasing now, after a cataclysmic decline in the early 1990s during the presidency of Fernando Collor de Mello. Collor, who resigned in 1992, held research in low esteem, ordering cuts across the board.



SOURCE: MCT



SOURCE: MCT

out because the parasite creates an autoimmune response, which a vaccine might exacerbate.

On display at the Caxambu meeting, which was largely sponsored by the Brazilian ministry of health, was the latest attempt to break this impasse: molecular genetics. Its centerpiece is an effort by Argentine, Brazilian, Chilean, Mexican, and Venezuelan biologists—along with collaborators in France and Spain—to sequence and map the parasite's entire genome, hoping to identify genes and their protein products that could serve as drug targets. Leaders of the project, including Mariano Levin of the Institute for Research in Genetic Engineering and Molecular Biology (INGEBI) in Buenos Aires and José Franco da Silveira of the Escola Paulista da Medicina in São Paulo, were among those making a report.

The aim, Levin said, is to create a physical map of *T. cruzi* by breaking its genome into well-defined, cloned segments and then sequencing the clones—an effort that he estimated will cost $1 million per year over 3 years. Members have already begun work using existing resources, but they've applied to WHO and other sources for additional funding, which they said will be essential to complete the project.

The project got under way last year, when Daniel Cohen of France's Centre d'Etude du Polymorphisme Humain (CEPH) invited a group of Latin American scientists to visit his lab. There they made clones of a strain of the parasite isolated by Brazilian biologist Zigman Brener. Copies of the clone libraries are now being distributed to interested researchers, while characterization and sequencing of these clones has begun at several labs—including among others Levin's in Buenos Aires, Bianca Zingales's at the University of São Paulo, and Wim DeGrave's at the Instituto Oswaldo Cruz in Rio de Janeiro, a top research center in Brazil formerly directed by Chagas.

Like earlier phases of the war against *T. cruzi*, leading biologists see the genome effort as a means of introducing the latest techniques in biology to Latin America. The Oswaldo Cruz Institute, for example, recently bought a sequencer and a Silicon Graphics workstation for its genome work. "We want the latest technology," says Hooman Momen, vice director and chief of molecular biology at the institute, which is also known as Fiocruz.

But the genome project isn't the only effort to bring up-to-the-minute biology to bear on the disease. Working independently of the project, Samuel Goldenberg, a French-trained investigator at Fiocruz, has cloned and expressed two genes for enzymes vital to the parasite. One plays a role in an RNA editing process that is unique to a type of organism called kinetoplasts; the other is important in energy metabolism. With Gregory Buck of Virginia Commonwealth University in Richmond, Goldenberg hopes to block the enzyme that acts on RNA, disrupting the parasite's replication, and he is also searching for ways to interfere with its metabolism.

Another strategy being pursued by Brazilian researchers in the campaign against *T. cruzi* is so-called rational drug design, in which researchers build on detailed structural knowledge of a pathogen's protein or enzyme to tailor a compound that can block it. One target of these efforts is a unique enzyme known as cruzipain that is essential for *T. cruzi*'s development within a host cell. Julio Scharfstein of the Instituto de Biofísica Carlos Chagas Filho in Rio was one of the first to isolate the enzyme, and James McKerrow of the University of California, Los Angeles, says he and his colleagues have "converged on the same molecule" and obtained images of its structure.

A broader effort in rational drug design is under way at the University of São Paulo at São Carlos, led by a British-educated structural biologist, Glacius Oliva. One of Oliva's graduate students has studied with crystallographer Wim Hol at the University of Washington, Seattle, learning how to crystallize a variety of trypanosome proteins and capture x-ray images of their structures. Oliva and his group intend to use these structures to design and test drugs that would inhibit *T. cruzi* enzymes.

These moves toward cutting-edge science are unnerving some other biologists in Brazil. One fear is that they will divert resources from other basic research—a concern stoked by WHO's recent announcement that it will be redirecting some of its grants for parasite research into genome studies. Others at the Caxambu meeting said they were concerned that WHO has created a two-tier approach. It is urging those who aren't in this elite circle of geneticists or who cannot compete in other special categories, such as drug development, to focus on what biologists in the developing world traditionally used to do—field research. To some, this sounds like "scientific colonialism."

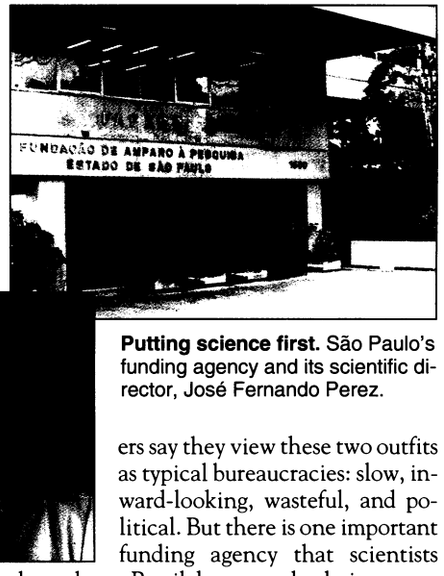
But to Fiocruz's Momen, the effort to build first-rate biology around Chagas disease is a fitting national ambition for Brazil. "We don't agree that there should be second-class research projects for the second world."

–Eliot Marshall

BRAZIL

Agency is Refuge in Funding Wilderness

SÃO PAULO, BRAZIL—First-World scientists may grumble about problems in getting funding. But their difficulties pale in comparison to those facing their counterparts in Brazil. Take the molecular biologist in Rio de Janeiro who thought he had hit upon the perfect way to beat Brazilian inflation, which in mid-1993 was skyrocketing at 50% per month. When this biologist recently got a grant check from the United States, he decided to cash it and stow tens of thousands of dollars in a safe-deposit box. That way, he thought, he could stabilize the value of his grant. It worked fine—until thieves broke into his bank and emptied all the deposit boxes, abruptly ending his project.



PHOTOS BY E. MARSHALL

Putting science first. São Paulo's funding agency and its scientific director, José Fernando Perez.

The case was exceptional, but many scientists in Brazil say that more conventional sources of funding don't serve them much better than that safe-deposit box did. Brazil's equivalent of the U.S. National Science Foundation—the Conselho Nacional de Desenvolvimento Científico e Tecnológico, known by its obsolete acronym, CNPq—focuses on a variety of targeted programs, stipends, and research institutes, leaving little money for investigator-initiated projects. The other big federal R&D funding agency, Financiadora de Estudos e Projetos (FINEP), favors high-tech ventures. Speaking privately, researchers say they view these two outfits as typical bureaucracies: slow, inward-looking, wasteful, and political. But there is one important funding agency that scientists throughout Brazil know and admire: a revolving fund for science and technology run by the state of São Paulo, called the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP).

Researchers love FAPESP because it is everything the other agencies are not: efficient, focused on quality, and run with scientists in mind. That tradition got started more than 30 years ago when progressive leaders in the state of São Paulo—Brazil's richest—decided to create a research trust fund that