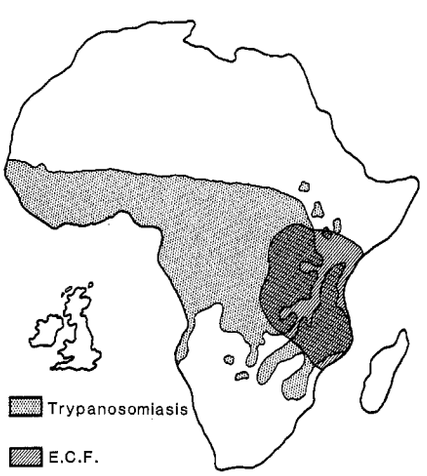
Nairobi Laboratory Fights More Than Disease

With a mandate to tackle two major animal diseases, an internationally funded laboratory has established a fine scientific reputation, but still has problems

Africa supports about 160 million cattle and 286 million sheep and goats; some of these are reared on large commercial ranches, but the majority provide the principal livelihood to subsistence farmers and pastoralists. Productivity—the lowest in the world—could be doubled, except for the prevalence of two widespread parasitic diseases, trypanosomiasis and a form of theileriosis called East Coast fever. Other forms of these two diseases are also important in large areas of Asia and South America. The International Laboratory for Research in Animal Diseases (ILRAD) was established in Nairobi, Kenya, in the early 1970's with the aim of succeeding with novel methods to control trypanosomiasis and theileriosis where traditional approaches had failed.



Cattle diseases in Africa

The map shows the geographical scope of trypanosomiasis and East Coast fever, with the United Kingdom as scale reference.

ILRAD's founders focused the laboratory's research on immunological control, for a long time the method of choice for bacterial or viral infections but still in its infancy in dealing with parasitic diseases. In the 7 years since the project began, ILRAD has encountered two major barriers to achieving its goal. The first is the work of nature, the second of man. ILRAD has yet to come to terms with either of them.

The first problem concerns the parasites themselves. Although the chances of immunological intervention with one of the diseases (East Coast fever) may now be promising, for the other they appear to be diminishing. The trypanosome is turning out to be so complex a piece of molecular machinery that conventional methods of immunization might well be impracticable. Intellectually, the challenge is ever more stimulating, but at a time when ILRAD's international backers are under increasing pressure to husband their budgets, the lack of imminent practical results could become embarrassing.

The second problem stems from ILRAD's status as an international research center in a Third World country. ILRAD is one of 13 centers that are embraced by the Consultative Group on International Agricultural Research (CGIAR), which has its secretariat at the World Bank in Washington. Like the other 12 centers, ILRAD has an international (mainly European and North American) team of researchers established in affluent surroundings in a relatively poor host country. A degree of envy of superior facilities and life-style is inevitable.

In addition, ILRAD is alone in the group of 13 centers in being mandated to do basic research only. The work of the laboratory does not involve the kind of technology transfer that, for instance, has given the International Maize and Wheat Improvement Center (CIMMYT), in Mexico, and the International Rice Research Center (IRRI), in the Philippines, such immediate and positive impact. As a consequence ILRAD is isolated from the activities of many national organizations and is perceived to be inward-looking and aloof. (The laboratory is now taking steps to overcome this problem.) Real affluence and apparent aloofness exacerbate each other in the onlooker's mind, and such an image does not make for political popularity in the international community.

Trypanosomiasis and East Coast fever are caused by blood-borne parasites that are transmitted to mammalian hosts by blood-sucking vectors, the tsetse fly in the case of trypanosomiasis and the tick in East Coast fever. Parasites travel from vector to mammal in saliva and from mammal to vector in ingested blood. Both diseases induce anemia and reduced productivity in infected mammals, and both frequently cause death.

The methods now available to combat these two diseases are inadequate in many situations. East Coast fever can be controlled by twice weekly dips in acaricides, which kill the tick vectors. This is expensive and impracticable for many African livestock producers, and the resistance of ticks to acaricides is an increasingly serious problem. Methods to control trypanosomiasis include regular drug treatment against the parasite and widespread spraying with insecticides to kill tsetse flies. Both approaches are expensive and neither is completely effective in areas of heavy infestation. Further drawbacks include increasing drug resistance and the possibility of environmental disturbance. "Assiduously carried out, such techniques can be successful," says Ross Gray, director general of ILRAD, "but in many cases the required standards of veterinary supervision and environmental control are simply unattainable." The great goal of an immunological approach is that a single inexpensive vaccination might give lifetime protection.

"The reason ILRAD was given its special mandate, and the reason we continue to hope for practical results, is that under certain conditions cattle acquire natural immunity to trypanosomiasis and theileriosis in the wild," says Gray. "Immunity is clearly possible, and our job is to find out if it can be carried out effectively, safely, and cheaply."

In its mammalian host, the parasite that causes East Coast fever, *Theileria parva*, passes first into white blood cells and then into red blood cells. Infected red blood cells reach the gut of the tick, a sexual stage is passed through, and eventually the parasite reaches the insect's salivary gland where tremendous multiplication occurs. One thoroughly infected tick contains enough parasites to kill 100 cattle.

Each year, half a million cattle are killed by East Coast fever in Africa, and yet it is already possible to immunize against infection. Cattle given an oxytetracycline-controlled infection of parasites develop a good solid immunity.

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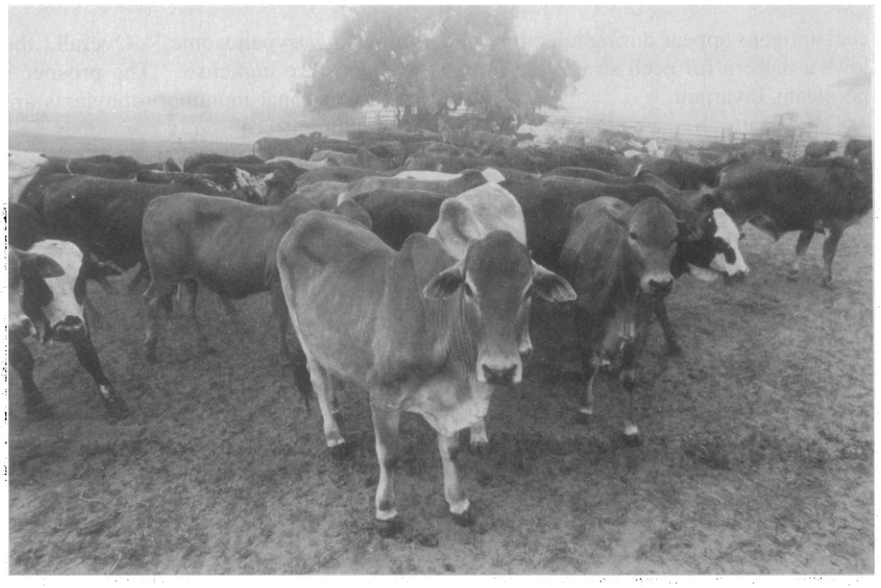
"There are a couple of difficulties with this approach," says Gray. "The first is a real danger of setting up a carrier state in immunized animals. And the second is that, inevitably, we are dealing with more than one strain of parasite, and as yet we don't know how many there are." This type of immunization requires close veterinary supervision, according to Gray, and is therefore too expensive for widespread application.

A second approach is to inject cattle with white blood cells that are infected with parasite at the macroschizont stage. Again, the induced immunity is very good, but there are problems. Immunity can be established with as few as 100 cells, but only if the injected blood is the animal's own. If the blood of another animal is used, then the dose has to be at least 10 million cells. Neither path is practical for routine immunization.

The more general issue with East Coast fever is determining more precisely the nature of the immune response. Antibodies are apparently involved in the sporozoite challenge, but to which antigens is not clear. Parasite-infected white blood cells provoke a cell-mediated response, apparently to a combined parasite and host antigen, the identity of which remains elusive.

"If we could only identify an effective antigen," says Gray, "we could perhaps shift some of the molecular biology emphasis from trypanosomiasis to East Coast fever." Through cloning the individual genes for important antigens it would be possible to manufacture large quantities of antigen for use in safe immunization. Gray is reluctant to "put a date on significant progress," but the first quinquennial review of ILRAD, reported at the end of 1981, anticipates a major breakthrough "within the next five years."

By contrast with East Coast fever, trypanosomiasis is more thoroughly understood and yet less accessible to immunological intervention. At its most basic, the problem is one of numbers. There are three species of cattle-infective trypanosomes, *Trypanosoma brucei*, *T. congolense*, and *T. vivax*, each of which has an unknown number of strains, although probably of the order of 20. Prospects for effective immunization decrease as such numbers increase. As if this were not bad enough, the trypanosome changes its antigenic coat at frequent intervals, thus evading a sustained host-immune response and making prospects for a vaccine yet dimmer. Each strain has a set of at least 100 coat antigens, and in some cases it may be as high as 1000. The order in which new



Cattle productivity is reduced by East Coast fever and trypanosomiasis

ILRAD is the best equipped laboratory in Africa

Part of ILRAD's elegant environs

coat antigens appear during infection follows a pattern for each strain, but is by no means invariant.

The means by which the parasite changes its clothes so neatly is yet to be elucidated, but some clues are emerging. Each parasite has a full complement of antigen genes, some of which are arranged in tandem. Expression of some of the antigens involves DNA rearrangement, including the production of a temporary extra copy. Rearrangements sometimes involve modification of part of the gene's structure. Scope for variation is large, but what imposes some degree of order on the sequence of gene expression is not yet known. What is known, however, is that the host's immune response does not induce the switch to a new coat; this appears to be a built-in property of the trypanosome.

"There's clearly no real hope of producing vaccines to all these variable antigens," concedes Gray, "but the one glimmer of hope we do have is that the metacyclic stage, in which they are transmitted by tsetse flies, has only a limited repertoire of antigens." When the parasites of any given strain enter the insect host, they emerge with just one of perhaps 10 to 15 possible metacyclic antigens. "It might be possible to produce a vaccine cocktail to this number of antigens," Gray suggests, "but you have to remember that even in one geographical area there are likely to be several strains of trypanosome." Overall, the problems are immense. "The prospects of conventional immunoprophylaxis are very slim indeed," Gray concludes.

Research facilities at ILRAD must be the best in Africa and rival those in any European or North American country. Molecular biology, biochemistry, and cell biology are bringing tremendous technical pressure to bear on the recalcitrant problems of trypanosomiasis and East Coast fever. "What sustains us," says Gray, "is that the technology is changing and so we no longer have to think in terms only of the traditional type of vaccine. We can think about making antigens and even manipulating the immune response to be more effective."

A combination of circumstances has conspired to make ILRAD's history somewhat turbulent. For a start, Gray is the laboratory's fourth director general in 7 years. The first, E. Sadun, died before he assumed office. Sadun's successor, James Henson, resigned during his second 2-year term. The third, Anthony Allison, was asked to resign after less than 2 years.

Compounding the inevitable practical problems involved in setting up a new laboratory in a Third World country, there have been accusations of administrative incompetence, racism, scientific poaching, and lack of leadership. ILRAD's beautifully manicured lawns were the site of more than one protest demonstration during Allison's directorship, for example.

Opinions differ, but the consensus in the laboratory is that Allison inherited an administrative quagmire in which no director could have survived, even one lacking Allison's strong and demanding personality. There is no doubt, however, that in spite of his curtailed directorship, Allison substantially enhanced the scientific standing of the laboratory.

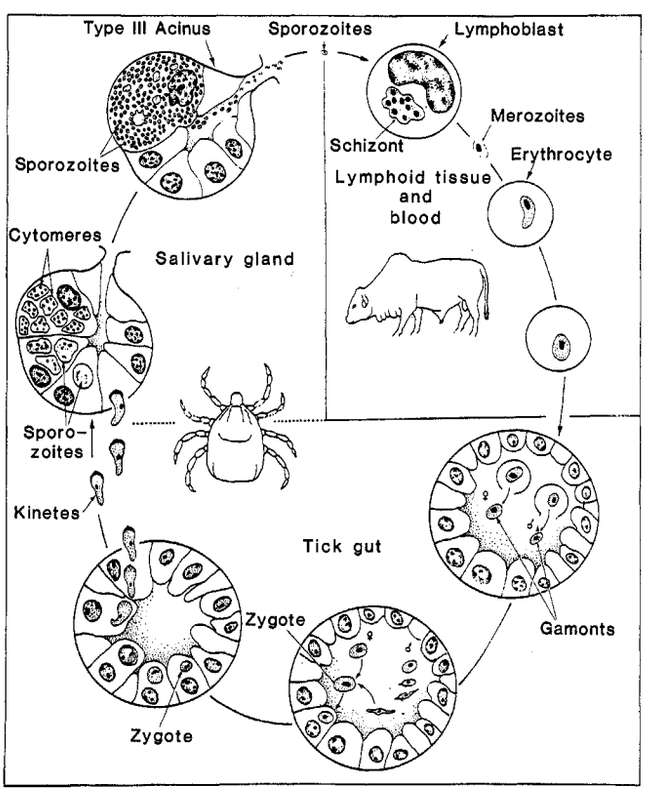
By the time Allison left ILRAD at the end of 1980, both the director of administration and the financial controller had also been asked to look for jobs elsewhere. Roger Rowe brought his extensive experience at another CGIAR center to the vacated position of director of administration, and some degree of order and stability began to settle on the beleaguered laboratory. Ross Gray, who had been a runner-up to Allison in the 1978 selection, took over as director general in November 1981.

Remarkably, throughout all the upheavals, the laboratory continued to produce good science and consolidated its growing international reputation. For instance, the work there has made it possible to cultivate two species of trypanosomes through their full life cycles. Four genes for trypanosome variable antigens have been cloned and the protein for one of them expressed. Unusual aspects of trypanosome surface antigen biochemistry have been discovered. The immune response to East Coast fever has been partially elucidated. Elegant electron microscopy has revealed important details of the *Theileria* life cycle. By any standards the list of achievements is long and impressive.

Gray describes ILRAD as "the trypanosomiasis and theileriosis university of Africa." That may well be so, but his appointment, particularly combined with Rowe's, is certain to shift the future direction of the laboratory, a shift that is probably crucial to ILRAD's long-term survival.

Gray says his first objective is to get his researchers to be more aware of the diseases they are supposed to be addressing. "The molecular biologists and biochemists are tremendous scientists," he says, "but they are not as familiar with the real field situation out there as I am." He is not suggesting they put on big boots and go tramping around cattle. "People can work at whatever level they like, as long as it is in some way related to producing an immunological solution. If people remember that just once a week it will be a start."

Although ILRAD's focus has been on



Life cycle of the tick

basic research, the laboratory is not as distant from the practical world as most research institutions are. There are not many facilities that have a P3 containment laboratory within hailing distance of 300 head of cattle. "This is one of the great strengths of the laboratory," says Max Murray, a senior ILRAD scientist. "We can go from the gene to the sick animal on the same site."

Nevertheless, Gray worries that most researchers' lack of contact with the field means that they have not concentrated their efforts in the right direction. "For instance, there's a great emphasis of work on *Trypanosoma brucei* as compared with the other species," says Gray, "and this happens to be the least important of the three in terms of disease." The researchers counter this by insisting that as *T. brucei* is the easiest to work with it provides a model from which to move on to the more difficult and more important species. Gray acknowledges the logic, but still finds it exasperating.

Gray plans to extend ILRAD's reach into the field in epidemiology and in the study of cattle that are naturally tolerant to trypanosomiasis. A small epidemiology project had already been planned when Gray arrived and was designed to try out some field tests that will help in identifying trypanosome strains. "We are doing this on a very carefully managed dairy ranch in the coastal region of Kilifi," says Murray, "where the density of tsetse flies is low and the number of strains of trypanosomiasis is probably limited."

Murray sees the ranch's relative freedom from disease as an advantage, as it simplifies the experimental procedure, whereas Gray sees the same attributes as a drawback. "I can understand their reason for doing it this way," says Gray, "but I would have preferred an area that had all the components of the trypanosomiasis complex, even though it is more difficult to analyze." There is clearly some tension between director and research staff, but no obvious sign of antagonism.

Researchers and herders have known for a long time that certain breeds of cattle are able to survive in tsetse areas where others cannot. One good example of these so-called trypanotolerant cattle is the West African N'Dama. Gray says that ILRAD will have to start looking at trypanotolerance much more seriously than hitherto and that existing collaboration with the International Livestock Center for Africa (ICLA) will have to be developed further. No one knows why N'Damas and other trypanotolerant animals are able to combat the trypanosome; their immune defenses might be better attuned to the parasite, or they might have a more general physiological resistance to the effects of infection. Clearly, a thorough understanding of the mechanism of tolerance might provide some important insights in combating the disease in susceptible animals.

"Alternatively," suggests Gray, "perhaps we should simply work out ways of screening for resistance and then carry out some good breeding programs." Gray knows that this would not be a completely satisfactory solution because no cattle are fully resistant to the disease. "It would, however, bring immediate results," he says, thus making the point that practical results are just as much the business of ILRAD as is understanding the fundamentals of the diseases.

"I hope they continue to do excellent work. But what my ministry is interested in is something practical—and fast."

Before joining ILRAD, Rowe spent several years at the International Potato Center in Lima, Peru. "Although we were based in Peru," says Rowe, "we had seven regional centers throughout the world. Our whole mode of operation was to look outward and to learn from people in the field." ILRAD does not work like this because of its mandate to do basic research. The laboratory is therefore much more isolated from its host country than is the case with other centers. If it is maintained, this insularity could endanger the laboratory's future, says Rowe.

Most CGIAR centers devote a good deal of effort to technical training so that the work of the center is disseminated to the countries that need it. "Centers should not be solving a host country's problems," says Rowe. "They should be helping a country get into a position to solve its own problems." Again, ILRAD is a little different because of the nature of its research. "Yes, we should be training people," says Luciana Rovis, one of ILRAD's founding scientists, "but we should be training the country's professors, not people who will treat sick animals." As things stand at the moment, Rovis is right. ILRAD is more attuned to turning out professors than people skilled in animal husbandry. But, contends Rowe, ILRAD must seriously contemplate extending its activities beyond the iron gates of its elegant research compound.

The recent quinquennial report noted the laboratory's minimal commitment to training. And at a meeting of centers and donors at the end of last year in Washington, ILRAD's training efforts were described as "pitiful." Gray says that he is conscious of the problem. "At the very least," says Rowe, "we should have a good working relationship with the people who would be responsible for deploying a vaccine, supposing we were to come up with one." To this end, Tony Irving, one of ILRAD's researchers, helped to establish last year a group known as the Nairobi Cluster, a collection of a dozen or so Kenyan institutions involved in work on trypanosomiasis and East Coast fever. "It's a start," says Rowe, "but there is a long way to go."

Until now the 13 centers supported through the CGIAR have not suffered financially, but the current downturn in the world economy is beginning to make donor countries and organizations measure their priorities. Progress and budgets will be considered at a meeting of center personnel and donors to be held in Paris at the end of May, and final pledges for the next financial year will be made at "Centers Week," to be held in Washington in the fall. A serious squeeze will be felt for the first time; ultimately funding for one or more of the centers may end. There would be a political outcry if, for instance, CIMMYT or IRRI were to be axed, but what about ILRAD? With so uncertain an impact on the world's problems, the laboratory might find few political supporters.

Ishmael Muriithi, a director in the Kenyan Ministry of Livestock Development, sums up ILRAD's achievement and predicament. Muriithi, a former board member of ILRAD, told *Science*: "I hope they continue to do excellent work. But what my ministry is interested in is something practical—and fast." Clearly, the emphasis on basic research written into the ILRAD mandate in 1973 could bring serious problems to the laboratory in these times of great economic stringency.—ROGER LEWIN

Portraits of a Parasite

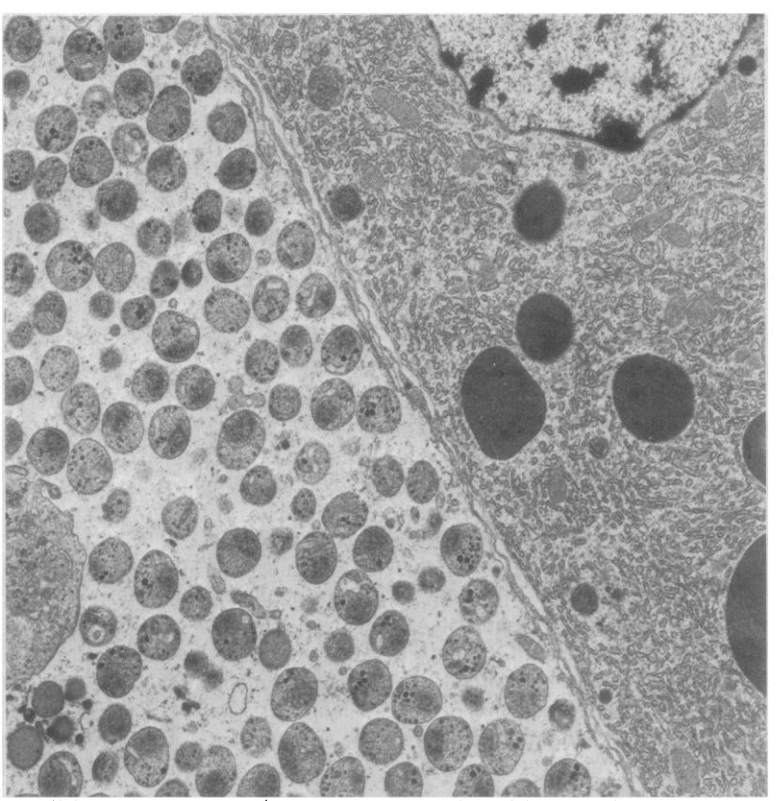
Don Fawcett, former chairman of Harvard's Anatomy Department, has been coaxing some excellent images from ILRAD's new electron microscope facility. With the help of Stephen Doxsey, and in collaboration with the Kenya Agricultural Research Institute, he has clarified some of the key events in the life cycle of *Theileria parva*, the causative agent in East Coast fever.

A crucial episode in the infection of cattle by *Theileria* is the entry of sporozoites into the mammalian host's white blood cells, a process that has been visualized only dimly in light microscopy and which was thought to have taken several hours. Fawcett's electron microscopy has revealed that the interaction of parasite and blood cell is highly specific, and probably involves the binding of ligand to cell receptor, with entry being complete in about 5 minutes (see photographs at right).

The infected white blood cells are unable to destroy the sporozoites because, by the time defensive lysosomes have formed in the cell, the invaders have rid themselves of the envelope of host cell membrane that is necessary for lysosome attack.

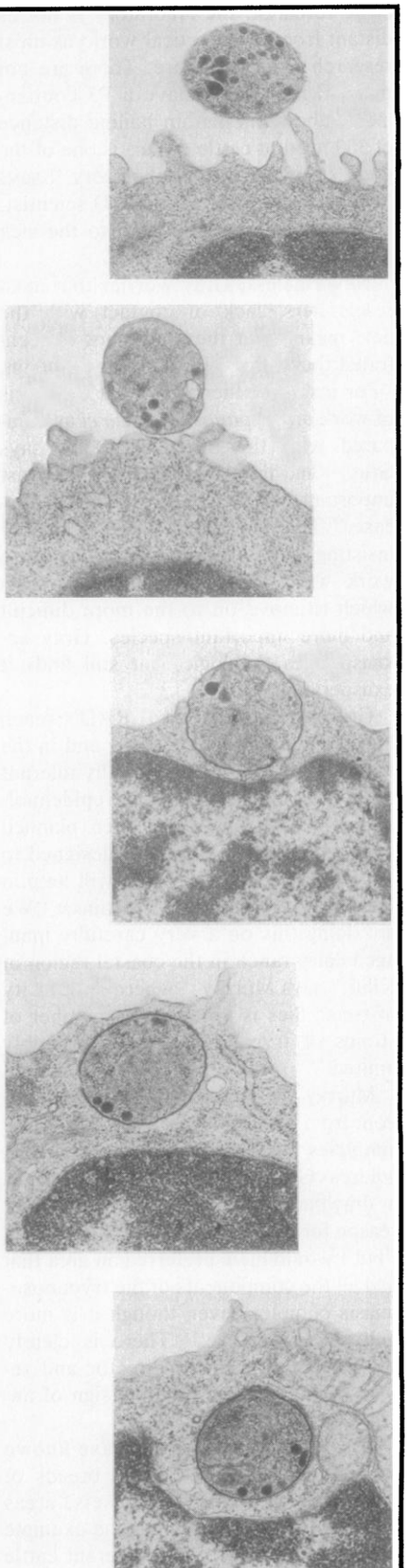
The parasite's odyssey through the tick vector takes it from the gut to the salivary gland, where sporozoites develop. Fawcett has shown that the parasite colonizes just one specific cell type in just one of three different groups of cells (acini) that make up the tick's salivary gland. Once invaded by a single parasite, the salivary gland undergoes what Fawcett describes as "one of the most dramatic transformations in cell biology."

The cell enlarges as the parasite forms a huge ramifying multinucleate bag; the metabolism is switched to serve the needs of the proliferating parasite, specifically in laying down massive stores of energy-rich glycogen; and eventually its cytoplasm is all but obliterated as upward of 50,000 sporozoites are formed by fission.—ROGER LEWIN



Parts of a normal cell (right) and a parasitized cell (left)

The extensive endoplasmic reticulum has disappeared in the parasitized salivary gland cell, which eventually will contain up to 50,000 sporozoites.



Sporozoite enters lymphocyte

The sporozoite binds tightly with the lymphocyte membrane and enters the host cell in about 5 minutes, a process that is not slowed significantly in the cold (sporozoite diameter: 1.5 micrometers).

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