ILRAD

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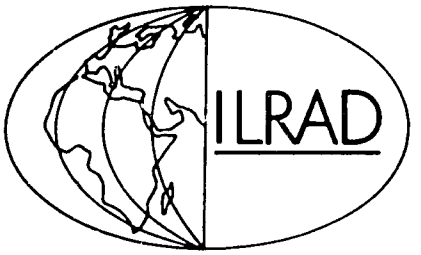

INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES

PROGRAM AND BUDGET

1984 - 1985

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1984-85 PROGRAM AND BUDGET

The Program and Budget for 1984-85 was prepared by the ILRAD staff and management, and was approved by the Board of Directors at the annual meeting in Nairobi on 23 - 25 March 1983.

This proposal constitutes a formal request to the Consultative Group on International Agricultural Research for donor support for 1984.

K F Wells
Chairman
Board of Directors

1984-85 PROGRAM AND BUDGET

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1. STATEMENT OF OBJECTIVES

The International Laboratory for Research on Animal Diseases (ILRAD) has the mandate to develop safe, effective and economically feasible control measures for livestock diseases which seriously limit world food production. Two parasitic disease complexes of major economic importance in many developing countries were chosen by the Consultative Group on International Agricultural Research (CGIAR) as the original focus of ILRAD's research program: trypanosomiasis and theileriosis, particularly East Coast fever.

East Coast fever (ECF) is a cattle disease which causes substantial losses in East and Central Africa. This form of theileriosis is caused by the protozoan parasite Theileria parva parva, which is transmitted by the brown ear tick Rhipicephalus appendiculatus. Mortality due to ECF is estimated at 500 000 animals a year. Other pathogenic Theileria species are a serious constraint on livestock production in extensive areas of North Africa, Asia and the Middle East.

ECF is controlled primarily by dipping or spraying cattle to kill the tick vectors. Proper pasture management helps keep tick populations low, and fencing and movement controls keep susceptible cattle away from animals which carry the disease. However, in areas of heavy tick infestation, cattle must be dipped or sprayed once or twice a week. Drugs to cure ECF are being developed by commercial firms, but neither frequent treatment with acaricides nor regular drug treatment is feasible for many African livestock producers, and tick resistance to currently available acaricides is potentially a serious problem.

The prospects for developing an ECF vaccine appear promising, since cattle which recover from natural or experimentally-induced infections show long-lasting immunity to the same or similar Theileria strains, even when the initial infection is mild. However, the epidemiology of the disease is complex, especially when buffalo are present as disease carriers. Numerous parasite strains need to be isolated and characterized and cross-immunity patterns determined. The major objective of ILRAD's theileriosis program is to improve current experimental immunization procedures which only protect against one or a small number of strains and which require the use of fully virulent parasites.

Different forms of trypanosomiasis occur over large areas of Africa, Latin America and the Middle East, affecting most types of domestic livestock, several wildlife species and man. In Africa, the major pathogenic species of trypanosomes for domestic ruminants are Trypanosoma congolense, T vivax and T brucei brucei. These are transmitted by several species of tsetse flies (Glossina spp) or occasionally by other biting flies. Trypanosomiasis occurs over about 10 million km^2 in tropical Africa, or roughly one-third of the continent. Affected areas often have relatively high rainfall and considerable potential for animal and crop production but due to trypanosomiasis very few ruminants are actually produced, and arable farming is limited by the absence of draught animals.

At present, measures available to control trypanosomiasis include regular treatment with trypanocidal drugs and reduction of tsetse populations by insecticide spraying. However, both of these approaches are expensive and neither is completely effective in areas of heavy tsetse density, high rainfall or dense vegetation. Further drawbacks include increasing drug resistance and the possibility of environmental pollution due to large-scale insecticide spraying. ILRAD's goal is to develop and introduce alternative control methods which are cheaper, more effective and without negative side effects.

Research to date indicates that immunological control of trypanosomiasis will be difficult. For one thing, domestic livestock are often exposed to more than one species of trypanosome and to a large number of strains within each species. A vaccine against one or a few strains will not necessarily confer immunity against others. What's more, even trypanosomes of one strain can display a large repertoire of different surface antigen types, and an infected animal cannot produce antibodies rapidly enough to match them all. This phenomenon - called antigenic variation - is probably the main reason why domestic livestock do not recover from trypanosomiasis or develop a natural immunity. It is also the main reason why it has not yet been possible to produce a trypanosomiasis vaccine. Research at ILRAD is concerned with several possible avenues of trypanosomiasis control, including studies of the parasites themselves, studies of the responses of different hosts and how useful host responses might be enhanced, and studies of trypanosomiasis epidemiology at several sites around Africa.

In addition to research on ECF and trypanosomiasis, ILRAD conducts an active training program. Training activities are designed to support the development of scientific and field personnel who can extend research on trypanosomiasis, theileriosis and other pressing veterinary problems - primarily in Africa, but also in other parts of the world. Scientists and technicians are trained to carry out the most effective disease control programs possible with the means currently available. At the same time, they are prepared to initiate new field programs when improved control methods are ready for introduction.

2. CURRENT STAGE OF DEVELOPMENT

A Quinquennial Review Panel of the CGIAR's Technical Advisory Committee (TAC) examined ILRAD's development in all areas in 1980 and presented a final report to the CGIAR at the end of 1981. The report expressed general satisfaction with ILRAD's scientific progress. The Panel recommended continuation of the laboratory research program with present areas of emphasis on theileriosis and trypanosomiasis. In line with the Panel's recommendations, laboratory research has been organized more formally into projects aimed at improved control of theileriosis and trypanosomiasis through the development of vaccines and the manipulation of host immune responses. ILRAD's field research program has also been expanded and strengthened as recommended by the Panel, and includes studies of ECF and trypanosomiasis epidemiology and collaborative research on the trypanotolerant cattle breeds of West and Central Africa.

As the prospects for improved control of theileriosis appear more promising in the short term, this program has been strengthened by the recruitment of scientific staff in immunochemistry, cellular immunology and epidemiology. Investigations have focused on different phases of the Theileria life cycle, with the aim of identifying the stages which are most likely to be vulnerable to immunological control. Laboratory research now concentrates on the development of immunization procedures based on antigens from two stages of parasite development - the sporozoite form which is transmitted to cattle by infected ticks and the schizont form which develops in the white blood cells of cattle. Scientific capacity in molecular biology is being developed to exploit any useful findings concerning parasite antigens which will require DNA technology. Another research area is theileriosis epidemiology, which began with the isolation and characterization of Theileria strains in a well-defined field situation at the Kenya coast and has led to successful pilot immunization trials. If results from field trials in Kenya justify further work along these lines and funds can be identified, these activities will be extended to other African countries affected by ECF. Collaborative research on the role of buffalo as ECF carriers is also in progress.

As the development of immunization procedures for trypanosomiasis appears to be a longer-term prospect, research on this diseases continues on a somewhat broader base. Areas of emphasis include possibilities of interfering with trypanosome metabolism and antigen synthesis and the genetic regulation of antigenicity. An additional scientist has been recruited to supplement expertise in the area of trypanosome antigenicity. Further staff are needed to strengthen the development of trypanosome culture techniques.

Research on host responses to trypanosomiasis is also emphasized, including immunological and nonimmunological aspects. Collaborative projects with the International Livestock Centre for Africa (ILCA) and other organizations focus on the mechanisms of resistance displayed by the trypanotolerant cattle breeds of West and Central Africa, and a collaborative project in Kenya

concentrates on resistance in several East African wildlife species. A trypanosomiasis epidemiology project is also in progress at the Kenya coast.

Increasing emphasis is being placed on the dissemination of ILRAD's research results to national and regional institutions concerned with the control of animal diseases in Africa. Opportunities for training scientists and senior technicians are steadily increasing as the scientific staff is enlarged to previously approved levels. A greater effort is also being made to include francophone African countries affected by trypanosomiasis and theileriosis in ILRAD's training and outreach programs.

In response to a recommendation made by TAC, an outline is being prepared of ILRAD's priority research areas for the next 5 to 10 years. This will provide the rationale for research plans outlined every 2 years in the program and budget documents. It will also help ensure that long-term objectives are kept in mind and will provide a yardstick for measuring progress towards attaining these objectives. As agreed by the TAC Quinquennial Review Panel, research efforts will not extend to other major animal diseases until at least one of the present research programs is satisfactorily concluded.

The construction program at ILRAD is largely complete. The research laboratories and supporting facilities are for the most part well equipped and functioning, and only minor alterations are required as laboratory functions and needs change. ILRAD provides offices for regional staff of four other CGIAR centres, and the arrival of scientists from a fifth centre is anticipated. Because this was not considered in the original building plans, additional offices are required. Experience in 1982 suggests that additional water-storage facilities, covered sheep and goat pens and a large-capacity incinerator are also needed. The construction of staff housing was finished in 1982, with the exception of a proposed house for the Director General.

A 15 000 ha cattle breeding ranch was purchased late in 1981 to provide an assured supply of cattle for experimental work. It is located on the Kapiti Plains about 80 km from Nairobi. The ranch has supplied calves and steers for ILRAD's research program since February 1982, and certain facilities on the ranch had to be renovated or remodeled to meet the new production requirements. Some of the urgent work was completed in 1982, but construcion of staff housing, spray races and fencing will continue in 1983 and 1984.

In 1981, a new management team took up appointments, including a Director General, a Director of Administration and a Financial Controller. A new Chairman of the Board also took office. At the beginning of 1983, the Director of Research position was filled. During 1982 and early 1983, 9 senior scientific and administrative staff members and 6 post-doctoral fellows were appointed, so as of March 1983, 88% of approved senior scientific and administrative positions were filled. This represents a considerable improvement over the situation in early 1982.

Another six senior positions need to be filled in 1983 to continue the steady build-up to full scientific strength. Recent findings on theileriosis have indicated a need for additional expertise in molecular biology, so current plans call for the recruitment of two molecular biologists to join the theileriosis program. A specialist is required in cell biology and parasite culture, and an immunologist/immunochemist will be recruited to work on parasite and cell surface antigens. One additional pathologist is needed to work on trypanosomiasis, and one parasitologist to strengthen research on T vivax and T congolense.

3. SUMMARY OF ACTIVITIES

THEILERIOSIS

Scientists at ILRAD have confirmed that cattle infected with ECF produce antibodies which are capable of neutralizing the sporozoite form of the parasite. To find out whether it will be practicable to develop a vaccine based on sporozoite antigens, it is important to determine whether one antigen or a small number of antigens is sufficient to stimulate immunity under field conditions, where animals are exposed to different ECF strains. If the relevant antigen(s) can be characterized precisely, they can probably be produced either by recombinant DNA technology or amino acid synthesis in sufficient quantities to provide starting material for an ECF vaccine.

Research was conducted in 1982 to isolate and characterize T p parva sporozoite antigens which are capable of stimulating an immune response. In one study, anti-sporozoite antibodies were taken from cattle infected with T p parva Muguga, a strain isolated earlier at the former East African Veterinary Research Organization. When tested in vitro and then in cattle, these antibodies were capable of neutralizing sporozoites from five other ECF strains isolated from different locations in East Africa. These results were unexpected, as some of these strains were not previously thought to be cross-protective. It was concluded that sporozoites of all five ECF strains produce either one common antigen or a common mixture of antigens which stimulates an immune response.

To identify these antigen(s) precisely, monoclonal antibodies were raised which respond to parasite antigens. Monoclonal antibodies are produced by creating hybrid cells from the fusion of spleen cells, which produce antibodies, and myeloma cells, derived from bone marrow. These hybrid cells are capable of growing and dividing in culture.

Purified sporozoite preparations are used as starting and screening material to select monoclonal antibodies which respond to specific sporozoite antigens. As part of this project, ILRAD immunologists have developed a new method for purifying sporozoites: material from infected tick salivary glands is separated by passage through columns of diethylaminoethyl cellulose, and the purity of the fractions obtained is monitored with the electron microscope.

Using sporozoites purified in this way, five highly specific IgM monoclonal antibodies were produced against T p parva Muguga. All five antibodies were found to recognize one common antigen. They also neutralized the infectivity of sporozoites of two other Theileria strains, indicating that these sporozoites all share a common antigen. Since these three strains were isolated from widely separated locations, it is possible that their common antigen may be found in other East African Theileria strains. This suggests that it might be possible to develop a vaccine based on this antigen which would protect cattle over a wide area.

Another technique was developed in 1982 to produce highly specific monoclonal antibodies which recognize sporozoite antigens. Monoclonal antibodies raised in mice are screened using a newly developed immuno-autoradiographic assay system which makes it possible to distinguish precisely which antibodies are responding to sporozoite antigens. This assay system was used to screen 360 monoclonal cell lines, and 5 were identified which showed strong antibody activity to T p parva Muguga sporozoites. The neutralizing activity of one of these antibodies has been confirmed by tests in cattle.

The sporozoite antigens which appear to stimulate a protective immune response are probably proteins, but their chemical nature is being investigated to rule out the possibility that they might be carbohydrates or lipids. Sporozoite antigens which are likely to be proteins are being identified according to their molecular weight using immunoautoradiographic techniques. The eventual aim is to produce these antigens by cloning in bacteria for use in immunization trials.

Research was also conducted in 1982 on the development of Theileria schizonts in bovine lymph cells and on the responses of the bovine host. Clinical cases of East Coast fever are characterized by the rapid proliferation of lymph cells infected with Theileria schizonts, followed by large-scale cell destruction. Scientists are investigating the uncontrolled growth and eventual destruction of parasitized cells to identify measures which could interfere with this process and thus prevent or modify the effects of the disease. ILRAD biochemists are screening the products of parasitized cells to identify growth-promoting factors as well as toxic products which could eventually cause cell destruction. Comparative studies of parasitized and non-parasitized daughter cells are being carried out to determine the factors and metabolic pathways involved in the maintenance of the parasitized state.

The terminal stage of ECF is characterized by massive cell destruction, but there is also evidence that cytotoxic (cell-killing) responses are involved in the acquisition of immunity. The difference between the two situations appears to lie in the specificity of the response. It may be possible to immunize cattle with non-infective preparations of parasitized cells which will stimulate specific cytotoxic responses, killing parasitized cells but not others.

In 1982, cattle were immunized successfully on an experimental basis with membrane preparations made from their own infected cells, while cattle immunized with membrane preparations from the cells of other animals did not develop immunity. This indicates that protective cytotoxic responses are directed against antigens on the cell membrane and that such responses are restricted by the host genotype. The next step will be to characterize the antigens which are recognized as targets by cytotoxic cells.

For some time now, scientists have been vaccinating cattle experimentally by infecting them with Theileria parasites and treating them with a long-lasting oxytetracycline antibiotic. This approach offers the best immediate possibility of improved disease

control while work continues on the development of a safe and effective vaccine based on antigens from sporozoites or schizont-infected cells.

One disadvantage of this 'infection and treatment' approach is that different strains of ECF are often not cross-protective: immunity conferred against one strain will not necessarily ensure protection against another. Secondly, not all strains can be controlled by oxytetracycline, so the intitial infection with live parasites may be fatal. Finally, buffalo are often chronic carriers of ECF, and the pattern of parasite strains is often very complex in areas where buffalo come into contact with cattle. Epidemiological studies at ILRAD focus on all these problems.

One important component of ILRAD's epidemiology program is the classification of Theileria strains and the identification of strains which do and do not cross-protect when used with current immunization procedures. By the end of 1982, a battery of 16 monoclonal antibodies had been raised which respond to bovine cells infected with Theileria schizonts. Based on profiles of monoclonal antibody responses, 22 T p parva stocks isolated from widely separated areas of Kenya, Uganda, Malawi and Zambia were categorized into three distinct groups. When cattle were immunized by infection and treatment against a stock in one group, they resisted reinfection by stocks of the same group, but not by stocks of different groups. The results of these cross-immunity trials suggest that it may be possible to categorize Theileria stocks and even develop hybrid strains as starting material for a vaccine which will protect animals over a wide geographic area.

Work is also in progress to improve experimental immunization methods. Techniques are being developed to measure doses of parasite material more accurately, and experiments have been carried out to determine the earliest age at which calves can be immunized safely. Cattle immunized experimentally are being kept in ECF endemic areas at three sites on the Kenya coast, and their responses to field challenge are being recorded. If these experiments are successful, they will demonstrate that stocks of T p parva can be isolated in an area, characterized in the laboratory and used to immunize cattle against the local strains. The next step would be to extend similar trials to other areas in Kenya and to other countries.

Collaborative studies aim at defining more precisely the relationship between T p lawrencei carried by buffalo and T p parva which infects cattle. In collaboration with the International Centre of Insect Physiology and Ecology (ICIPE), ILRAD scientists are also analysing tick saliva and bovine responses to saliva and other tick products which might be manipulated to increase resistance to ECF.

TRYPANOSOMIASIS

Beginning with T b brucei, techniques have been developed at ILRAD to maintain trypanosomes in tissue culture systems through all the stages of their lifecycle. Recent work has focused on extending

these techniques to the cultivation of T congolense and T vivax. Once a system for maintaining trypanosomes is proven successful on an experimental basis, it can be standardized and used to produce large numbers of parasites for biological, biochemical and immunological research.

Using the culture system established for T b brucei, two simple in vitro tests were developed in 1982 to screen the effectiveness of trypanocidal drugs. This work is being continued in 1983. A simplified method devised in 1981 for propagating T congolense metacyclic forms in vitro has now been standardized and put into general use. This is the stage of the parasite lifecycle which is transmitted to livestock by infected tsetse flies. Six populations of T congolense are now maintained in vitro. Work with T vivax concentrates on two stages of parasite development: the coated trypomastigote which develops in the mammalian bloodstream and the uncoated trypomastigote which is presumably equivalent to the next stage of development after the trypanosome is ingested by a tsetse fly. Morphological changes in bloodstream forms of T vivax have been observed for the first time.

Other research on trypanosomes in 1982 concentrated on the genetic basis of antigenic variation and on the structure and synthesis of the variable antigens forming the surface coat. Work also focused on key transitions in the parasite life cycle.

The expression of different surface antigens is associated with rearrangements in the parasite DNA. ILRAD scientists have observed two types of gene rearrangement in T b brucei, one based on expression-linked duplication and the other, more unusual, rearrangement involving the appearance of a minichromosome.

Biochemists at ILRAD are investigating how these antigens are synthesized and moved to the cell surface. Protein molecules are synthesized on the rough endoplasmic reticulum and are transported by the smooth endoplasmic reticulum to the Golgi apparatus. At some point, important carbohydrate side chains are added. Purified fractions of Golgi and rough and smooth endoplasmic reticulum have been produced, and the enzymes involved in the synthesis of the antigens are being characterized. A full understanding of this process could point to interventions which would disrupt the stability of the surface coat and render the trypanosome susceptible to host defences.

Several studies have been carried out on the changes which trypanosomes undergo at different stages in their lifecycle. These transitions might serve as points of intervention to disrupt the development of the parasites. Evidence was obtained in 1982 that all three major trypanosome species differentiate in the mammalian bloodstream from a rapidly-dividing to a nondividing form. One protein, calmodulin (CaM), has been detected which may play a role in this differentiation process. Trypanosome CaM appears to differ biochemically from mammalian CaM, so it may be possible to treat animals to inhibit trypanosome CaM and disrupt the parasite lifecycle without interfering with the system of the host.

Improved techniques are being developed to identify different trypanosome species and populations of similar genetic background, known as serodemes. Monoclonal antibodies have been raised against T congolense and T b brucei parasites and the antigens recognized by these antibodies have been isolated using immunoadsorbent columns. Purified T vivax antigens are now being prepared using the same technique and antigens from all three species are being characterized biochemically. Antigens specific for different trypanosome species will eventually be used in diagnostic assays to characterize infections in the field.

Another system is being developed on an experimental basis to identify trypanosome species and serodemes by analysing parasite DNA. This approach has been applied successfully to distinguish T b brucei from T b rhodesiense, one of the trypanosome species which cause human sleeping sickness. However, long and complex purification procedures are required to isolate DNA, which limits the usefulness of this system under field conditions or for large-scale studies. For this reason, a simplified laboratory technique is now being developed. Another experimental method for identifying T congolense and T b brucei serodemes involves priming animals by infection with one serodeme, treating them, and then infecting them with various field isolates and monitoring responses in the skin.

An improved serological system was adopted in 1982 for analysing tsetse blood meals to identify the animal species on which the flies have fed. Several techniques were evaluated for this purpose, but the enzyme-linked immunosorbent assay (ELISA) proved to be the most useful, particularly for studies under field conditions. Using this system, samples can be analysed quickly and only small amounts of material are required. This work was carried out in collaboration with the University of Nairobi's Faculty of Veterinary Medicine.

A number of studies at ILRAD focus on the complex interactions between trypanosomes and their hosts. Research concentrates on factors which affect host antibody production and on other host responses which play a role in resistance to the parasites.

Animals infected with trypanosomes appear to produce antibodies largely against the variable surface antigens. As infection progresses, antibodies to certain variable surface antigen types appear in recurring peaks. Experiments are in progress to isolate the variable antigen types responsible for this pattern of antibody production.

There is some evidence that cattle which manage to resist trypanosomiasis produce antibodies against other parasite antigens which are less variable than those on the surface coat. Antigens recognized by resistant animals have been isolated and are now being purified for possible use as the basis of an experimental vaccine.

However, the key factor in resistance to trypanosomiasis appears to be the capacity to control parasite growth rates. Scientists at ILRAD are studying host reactions which may limit trypanosome growth and development in the skin at the site of an infected tsetse bite

as well as in the bloodstream. Studies also cover responses in the central nervous system at later stages of infection.

A hard swelling, called a chancre, develops in the skin at the site of a tsetse bite when the fly is infected with T congolense, T b brucei and some strains of T vivax. This swelling represents the host's first response to the parasite. When resistant wild animals, such as buffalo, eland, oryx and waterbuck, are bitten by infected tsetse flies, they develop smaller chancres than susceptible livestock. Parasites also appear much later in the bloodstream of these wild species, which suggests that resistance is related to the capacity to control parasites in the skin before they reach the bloodstream. Scientists at ILRAD are studying the skin response in detail, concentrating on the identification of biologically active molecules in the chancre which may be of parasite or host origin.

For T b brucei and possibly for other trypanosome species, the level and duration of parasitaemic waves in the bloodstream appear to be related to parasite differentiation from actively-dividing to nondividing forms. In the case of T b brucei, host antibody production only begins when the parasites differentiate to the nondividing form. Earlier research showed that some host factor plays a role in this process, because differentiation occurs at different times in different host species and strains, even when the parasite population is genetically identical.

Detailed studies carried out in 1982 confirmed that host resistance to T b brucei is related to the ability to control parasite growth rates in the bloodstream, which is in turn related to the timing of parasite differentiation. Several studies have indicated that some host factor is involved other than antibody production. Genetic experiments in mice suggest that this factor is controlled by a complex co-dominant gene system.

Cattle and goats infected with T b brucei sometimes develop a fatal illness characterized by clinical abnormalities of the central nervous system, similar to those observed in human trypanosomiasis. In 1982, brain lesions were observed both after primary T b brucei infections and after treatment with the trypanocidal drugs normally used for livestock, which cannot cross the blood-brain barrier. These infections in livestock provide a good in vivo model for the study of cerebral trypanosomiasis in humans and for testing the effectiveness of drugs used for the human disease.

Comparative studies on host resistance to trypanosomiasis focus on different breeds of cattle, sheep and goats and species of wild animals. One goal is to find genetic markers which can be used to identify relatively resistant animals for livestock improvement programs. A better understanding of how some animals resist trypanosomiasis may also suggest promising interventions for increasing the resistance of others.

ILRAD scientists are collaborating in a research and training network with colleagues at ILCA, ICIPE and national organizations around Africa, focusing on the productivity and resistance to trypanosomiasis of different livestock breeds under clearly defined

management regimes and levels of trypanosomiasis risk. The network covers sites in 13 countries, concentrating primarily on the recognized trypanotolerant breeds of West and Central Africa. ILRAD's role in most of these areas is to provide training and supervision for the animal health and tsetse survey components. In The Gambia, plans are well advanced to initiate research on animal health and productivity in trypanotolerant N'Dama cattle. The ILCA/ILRAD component of this work will be carried out with financial support from the European Economic Community (EEC).

In addition to collaborative studies in West Africa, there is an urgent need for research on trypanotolerant livestock at the ILRAD site at Kabete, where full use can be made of specialized staff and modern equipment and facilities. Because animal health restrictions prevent the importation of live trypanotolerant cattle into Kenya, fertilized ova have been obtained from N'Dama cows in The Gambia and implanted in Boran heifers at the Kapiti ranch. Calves are expected in March 1984.

Initial studies have shown that some indigenous Kenyan livestock breeds show a degree of resistance to trypanosomiasis, though probably much less than the resistance shown by the recognized trypanotolerant breeds. The role of chemotherapy in the acquisition of resistance to trypanosomiasis will be examined in field situation in Kenya and in other African countries in collaboration with ILCA. In collaboration with the Kenya Veterinary Research Laboratories, ILRAD scientists are also studying the mechanisms of resistance to trypanosomiasis displayed by East African wildlife species. This wildlife research project is funded by the Dutch Government.

An epidemiological survey was conducted in 1982 in an area near the Kenya coast with a low level of trypanosomiasis risk and little movement of livestock or game animals. This included a preliminary tsetse survey, blood sampling from over 5000 cattle in the area and monitoring infection in 20 tracer cattle brought into the primary tsetse focus site. Sixty-one blood stabilates have been obtained from infected animals: these are being analysed to determine the number of different serodemes involved in the infections, to monitor the appearance of protective host antibodies and to assess the role of chemotherapy in the acquisition of immunity.

RESEARCH SUPPORT

Tsetse Laboratory

Breeding colonies of 8 tsetse species are maintained at ILRAD, providing a good representation of the 30 tsetse species and subspecies found in tropical Africa. The colony of *G morsitans centralis* provides material for most of ILRAD's trypanosomiasis research. Colonies of *G m morsitans*, *G austeni*, *G palpalis*, *G fuscipes fuscipes*, *G brevipalpis* and *G pallidipes* were initiated in 1982. These tsetse species are all being evaluated in terms of their capacity to transmit *T vivax*, *T congolense* and *T b brucei* to ruminants. In addition to supporting research at ILRAD, the Tsetse

Laboratory supplied 16 613 puparia and adult flies in 1982 to research centres in Kenya, Togo, Zambia, Austria and Switzerland.

Tick laboratory

ILRAD's tick laboratory maintains breeding colonies of two strains of Rhipicephalus appendiculatus, the vector for T p parva and T p lawrencei, and one strain of Amblyomma variegatum, the tick species which transmits T mutans. The tick laboratory routinely provides ILRAD scientists with infected and uninfected ticks, tick salivary glands and other organs and tick-derived Theileria stabilates. Staff from the tick laboratory also test the infectivity of Theileria stabilates and monitor the course of experimental infections in cattle.

Farm animal production

Scientists at ILRAD used about 400 cattle for research projects in 1982. About 85% of these were used for ECF research and 15% for work on trypanosomiasis.

The cattle ranch which ILRAD purchased at the end of 1981 included over 2000 Boran (Bos indicus) cattle, with a breeding herd of about 750 mature cows and 220 heifers. In 1982, the ranch supplied 335 calves and steers to ILRAD's research program. By the end of the year, the breeding herd had grown to 786 mature cows and 595 heifers.

In addition to the animals supplied by the Kapiti ranch, 26 calves were bred at ILRAD and 26 Bos taurus cattle were purchased from other ranches in Kenya for experiments which require these breeds. With the purchase of the ranch and expansion of cattle holding facilities at ILRAD, a steady supply of animals was assured for most research projects throughout the year. The supply of Bos taurus cattle was interrupted for a period, however, when movement restrictions were imposed due to an outbreak of foot-and-mouth disease.

Most research work involving cattle was carried out at the ILRAD site in Kabete, but 28 animals were taken to Kilifi as part of the ECF epidemiology project. Ten Zebu cattle were also used for a collaborative trypanosomiasis research project, housed at the Kenya Veterinary Research Laboratories near ILRAD.

ILRAD scientists used about 400 small ruminants in 1982, comprising 370 goats and about 30 sheep. These animals are of local East African breeds, purchased from the area around ILRAD. In 1982, 95% were used for trypanosomiasis research. The screened housing available at ILRAD for trypanosomiasis experiments can accommodate 300 small ruminants at one time. These facilities were used to capacity in 1982. Expansion of the trypanosomiasis epidemiology program may require the construction of additional fly-proof accommodation.

Laboratory animal production

The laboratory animal unit maintains breeding populations of mice, rats and rabbits, and small colonies of guinea pigs and gerbils. The mouse breeding program expanded from 5 inbred strains in 1981 to 12 strains in 1982, including a random-bred strain of nude mice. Mice are used primarily to study the course of trypanosomiasis infections and mechanisms of resistance. Nude mice are particularly valuable in experimental work because their cell-mediated immune responses are inhibited.

One inbred rat strain is maintained, primarily to produce the trypanosomes required by ILRAD scientists. A strain of larger rats was introduced in 1982, replacing the strain used previously. Rabbits are bred to support the tsetse and tick colonies and to produce sera for research and diagnostic purposes. A new line of full lop-eared rabbits was introduced in 1982.

Altogether, 43 392 mice, 25 075 rats and 450 rabbits were prooduced in 1982, representing a substantial increase over previous years. In addition to supplying nearly all the laboratory animals used at ILRAD, the breeding unit donated animals to ICIPE, the Kenya Trypanosomiasis Research Institute, the University of Nairobi, the National Museums of Kenya and a Food and Agriculture Organization (FAO) project in Zambia.

Clinical and diagnostic services

The diagnostic laboratory carries out several types of routine analysis for the research laboratories and animal production facilities at ILRAD. In 1982, a total of 26 014 samples were received for analysis, more than twice the number received in 1981. These included 17 246 samples for serological, 5473 for bacteriological, 2284 for haematological and 1011 for helminthological analysis. This substantial increase was due to the expanded ECF epidemiology project at the Kenya coast and the increase in cattle held by ILRAD with the acquisition of the ranch at Kapiti.

Serological screening and titration were provided for antibodies to six bloodstream parasites found in cattle - *T p parva*, *T mutans*, *T b brucei*, *T congolense*, *T vivax* and *Anaplasma marginale*. Most routine screening for the *Theileria* and *Trypanosoma* species was by the indirect fluorescent antibody test (IFA). The ELISA was also used to screen bovine sera for the three species of trypanosome. The card agglutination test was used to screen sera for *A marginale* antibodies.

During the year, 210 post-mortem examinations were carried out in the course of trypanosomiasis and ECF investigations. Of these, 111 cattle were examined, plus 88 goats, 7 sheep and 4 animals of other species. Cases of salmonellosis, infectious bovine rhinotracheitis and muscular dystrophy were encountered in cattle, as well as the diseases under study. Urolithiasis and coccidiosis were found in goats.

In addition to services provided to ILRAD scientists, the diagnostic laboratory continued to provide assistance to other centres and projects in developing countries. T p parva schizont antigens, positive and negative control sera, and goat antibovine IgG and IgM conjugate were provided to the National Veterinary Laboratory in Uganda and to FAO projects in Burundi and Mozambique. Tests for Theileria and Trypanosoma antibodies were performed for a German (Federal Republic) technical aid project in Somalia.

Radioisotope laboratory

ILRAD scientists carry out experiments requiring the use of radioactive tracers under controlled conditions in the radioisotope laboratory. Often experimental work is initiated here which can be continued in the other laboratories once the radioactivity of the material has returned to safe levels.

The staff of the radioisotope laboratory supervise the use of radio chemicals and the management of radioactive wastes. They also conduct a continuous radioactivity monitoring program, covering staff members, laboratory areas and the environment at the ILRAD site.

Central core laboratory

The central core laboratory is a specialized support facility where cell culture media, solutions and purified water are prepared as required for ILRAD's research activities. The central core also includes facilities for washing and sterilizing all the glassware used in the laboratories.

Library

The ILRAD library maintains a specialized collection of scientific literature, comprising about 2400 books, subscriptions to 198 periodicals, and a selection of maps and materials on microfiche. Periodicals are received every week covering topics in the fields of parasitology, immunology, biochemistry, entomology, cell biology and veterinary medicine. These are made accessible to scientists in other research institutes in the area as well as to ILRAD staff. Many are not available in any other library in Kenya.

The library acquires material requested by ILRAD scientists through a regional inter-library loan system or by purchase from the British Library Lending Division. Articles and conference papers published by ILRAD staff members are held in the library, and copies are distributed on request.

TRAINING AND OUTREACH

Scientists, senior technicians, post-graduate students and post-doctoral fellows come to ILRAD for specialized training on an individual basis. They work closely with the scientific staff within the scope of ILRAD's research mandate. Opportunities for

this type of individual training are steadily increasing, based on availability of supervisory staff, laboratory space, equipment and accommodation. At the same time, at least one major international training course is held at ILRAD every year, and, within budgetary limits, a number of scientists from developing countries are sponsored to attend appropriate training courses offered by other institutions.

In 1982, 14 scientists and technicians came to ILRAD for specialized training, for periods lasting from 1 week up to 1 year. Thirteen came from nine African countries and one from India, and all were employees of national universities, veterinary laboratories or research centres. Their training programs were planned on an individual basis according to the needs of their home institutes.

Three Kenyans from the Ministry of Livestock Development received training, two in basic haematological and parasitological techniques and one in the dissection of Theileria-infected ticks. One staff member from the Uganda Trypanosomiasis Research Organization was trained in histological techniques and another learned methods for maintaining laboratory animals. Two Zambians from the National Council for Scientific Research came to ILRAD to learn parasitological techniques relating to ECF. One Zairean from the Laboratoire de Parasitologie of the Université Nationale de Zaire was trained in parasitological and diagnostic techniques related to trypanosomiasis, and a Zimbabwean from the Veterinary Research Laboratory learned how to maintain Theileria parasites in culture. The head of the Parasitology Department at Addis Ababa University in Ethiopia received training in parasitological techniques related to trypanosomiasis, and a scientist from the Nigerian Institute for Trypanosomiasis Research learned techniques for maintaining T vivax in culture. A staff member from the Laboratoire Central Vétérinaire in Mali was trained in immunological and parasitological techniques, and a member of the Immunology Department of the University of Yaoundé in Cameroon studied antigenic variation in trypanosomes, supported by the World Health Organization (WHO). From India, a professor of parasitology at Punjab Agricultural University received training in basic cell culture and specific techniques for cultivating Theileria parasites in vitro.

Fifteen post-graduate students worked with ILRAD scientists in 1982 on projects closely linked with ILRAD's research program. These students are normally registered in a higher degree program at the University of Nairobi or another university. They work at ILRAD from 1 to 3 years and submit their projects as part of their degree requirements. In 1982, 10 students in this category came from Kenya, 2 from Uganda, and 1 each from Rwanda, Australia and Belgium.

Post-doctoral fellows are selected internationally, though emphasis is placed on recruiting well-qualified young scientists from African countries. They normally spend 2 years at ILRAD, and their work is expected to contribute directly to the research program. In 1982, 11 post-doctoral fellows worked at ILRAD, including 2 from Germany (Federal Republic), 2 from the UK and 1 each from Kenya, Uganda, Zaire, India, Belgium, Japan and the USA.

Altogether in 1982, 40 students, scientists and technicians received training at ILRAD on individual basis, 8 more than in 1981. Thirty of these trainees were from African countries, including five from francophone Africa.

In addition to training opportunities offered to individuals, a 6-week training course was held at ILRAD in October and November 1982 on 'The Use of Immunological Methods in the Diagnosis of Protozoan Diseases'. Fourteen participants attended this course from 10 African countries, including 5 from Kenya and 1 each from Liberia, Mali, Nigeria, Senegal, Sudan, Tanzania, The Gambia, Uganda and Zambia. The participants reviewed basic biochemical and immunological concepts with ILRAD scientists and learned how to produce antibodies, isolate and purify immunoglobulins, assess specificities, prepare antigens and cultivate different species of parasite. They were also taught to perform a range of serodiagnostic tests, with emphasis on the diagnosis of protozoan parasites.

ILRAD scientists helped conduct a 10-week training course in collaboration with colleagues from ILCA and ICIPE. This was the first of a series of training sessions for field staff taking part in the ILCA/ILRAD livestock productivity and trypanotolerance research network. The course covered the extraction, analysis and interpretation of data on animal production in areas under trypanosomiasis risk. Four scientists participated in this session, one from Ethiopia and three from Zaire, and one participant from Nigeria received the same training on an individual basis earlier in the year. A training manual, 'Livestock Productivity and Trypanotolerance', was prepared in English and French in connection with the course.

ILRAD's training program provided support for three scientists to attend a 1-week Workshop on Trypanotolerance and Animal Production, held in Avetonou, Togo in May 1982. The workshop was sponsored by the Centre de Recherche et d'Elevage, the Government of Togo and the Agency for Technical Cooperation and the Foundation for International Development of the German Federal Republic. One of the participants sponsored by ILRAD came from Senegal, one from The Gambia and one from Uganda.

Scientists at ILRAD published 35 articles in international journals, 3 chapters in books and 3 papers in conference proceedings during the course of the year. The 1981 annual report was prepared in English - and for the first time in French - and a series of brochures was issued in both languages describing ILRAD's research and training activities and listing all scientific publications. In addition, ILRAD's communications staff prepared press releases and articles for outside publication and arranged for coverage of ILRAD's research activities on Kenyan television.

The regular seminar series continued in 1982, with sessions held every 2 weeks. These were well attended by scientists from ILRAD, the University of Nairobi and other national and international research centres in the area. ILRAD staff members also participated in numerous international conferences and gave lectures on

trypanosomiasis and theileriosis research to participants in training courses sponsored by other organizations.

ILRAD's research and training activities were presented to a large audience in September 1982 in an exhibit prepared for the Nairobi International Show. This show is sponsored every year by the Agricultural Society of Kenya. Thousands of people from all over Kenya visited ILRAD's exhibit.

ILRAD staff members have played a key role in setting up the Nairobi Cluster, an informal organization of university departments, government laboratories and research institutes working on trypanosomiasis and ECF. Eleven institutions in the Nairobi area are members. The Cluster issued a newsletter in 1982 and sponsored a scientific meeting on 'Strategies and Recent Developments for the Control of Trypanosomiasis and East Coast Fever', held at ILRAD. The meeting was opened by the Minister for Livestock Development, the Hon Mr. Paul Ngei. Participants included scientists, government officials, livestock producers, veterinarians and representatives of drug companies.

Research projects were carried out in collaboration with agencies and organizations in many parts of the world. In Kenya, ILRAD scientists were involved in research and technical collaboration with the Ministry of Livestock Development, ICIPE, the Kenya Veterinary Research Laboratories, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Kenya Trypanosomiasis Research Institute, the OAU's Inter-African Bureau of Animal Resources, ILCA, the University of Nairobi's Faculties of Medicine and Veterinary Medicine and the Clinical Research Centre of the Kenya Medical Research Institute. Research was also conducted in 1982 in collaboration with the Centre de Recherche et d'Elveage in Togo, the Centre de Recherches sur les Trypanosomiases Animales in Upper Volta and the national veterinary laboratories of Burundi, Malawi, Mozambique, Rwanda, Tanzania, Zambia and Zimbabwe. Outside Africa, ILRAD scientists collaborated with colleagues at the Prince Leopold Institute for Tropical Medicine, the Vrije Universiteit Brussel and the Libre Université de Bruxelles in Belgium, the Swiss Tropical Institute, Cambridge, Glasgow and Edinburgh Universities in the UK and the Universities of California, Iowa and Washington State in the USA.

4. 1982 PERFORMANCE

Table 1. 1982 BUDGET SUMMARY (US $'000)

	Final Revised	Actual
Core operations	8 189	7 286
Contingency	150	166
Capital	1 350	1 366
Total	9 689	8 818
Less funds brought forward	(2 692)	(2 693)
earned income	(24)	(100)
Net requirement	6 973	6 025

Total expenditures for core operations in 1982 were US $700 000 less than the final revised estimate. This underutilization of funds was partly due to the fact that fewer international staff were employed than anticipated. In addition, the effects of inflation in Kenya were reduced by two devaluations of the Kenya shilling during the year. Another significant factor was the success of administrative efforts to control costs.

TAC has established a ceiling for ILRAD of 51 senior scientific and administrative positions. Of these, 37.9 manyears were used in 1982, with 34.5 supported entirely by ILRAD. Fourteen new staff members were recruited during the year, only two less than anticipated. However, since most of these staff members took up their appointments near the end of the year, total manyears in 1982 did not differ significantly from the 1981 level.

The recruitment of international staff was emphasized in 1982 so ILRAD would be nearer full strength by the beginning of 1983. However, TAC and ILRAD management anticipated that funding would only be available for 44 positions in 1983, so not all positions which were open in principle could actually be filled.

Since ILRAD was established, a number of non-core-funded scientists have contributed to the research program, including associate experts, visiting scientists and post-doctoral fellows. These scientists contributed 2.4 manyears in 1982 and will provide 3.5 manyears in 1983. Normally they receive salaries and travel

support from other sources. ILRAD provides research facilities and in some cases contributes towards living costs by providing on-site housing.

The 1982 operating costs were less than the final estimates for all research units except Central Core. Expenditures were greater than estimated in Central Core because all purchases of radioisotopes were centralized and charged to this unit. This procedure provided better control of the purchase and use of these highly perishable chemicals and reduced loss and waste. Individual laboratory budgets were actually reduced by a greater margin than the increase in costs for Central Core, resulting in a net saving overall.

Expenditures for Food and Housing were well below the anticipated level because income was credited against cost, instead of being listed separately as earned income. Thus, the net, rather than gross costs are shown. This procedure, which was initiated in 1981, was more detailed and complete in 1982, as this section has moved towards operation on a self-sustaining basis.

Total capital expenditure in 1982 was slightly higher than planned. Actual expenditures for laboratory equipment and construction exceeded projections for certain items. These were partly compensated by lower expenditures for other items.

During 1982, US $130 000 was spent for capital development of ILRAD's ranch, Kapiti Plains Estate Ltd. A manager's house and a cattle-holding facility were constructed and water supplies were improved. To improve tick control on the ranch, a new spray race was installed and an existing one was renovated. In addition, a long-term internal fencing project was initiated, and a tractor and two trailers were purchased. Operating expenses (not including capital or depreciation) were US $176 000 for the year. Income from the sale of livestock to ILRAD and to commercial buyers totaled US $173 000.

ILRAD ended 1982 with nearly US $1 million in funds to carry forward. In addition to the underutilisation of funds mentioned previously, earned income was greater than expected during the year. This was partly because a positive cash flow made it possible to earn interest and also because the Kenya Government for the first time permitted the sale of cattle from the ILRAD site at Kabete.

5. 1983 PROSPECTS

Table 2. 1983 BUDGET SUMMARY (US$'000)

	1982 Actual	1983 Current Estimate
Core operations	7 286	8 562
Contingency	166	170
Capital	1 366	770
Total	8 818	9 502
Less funds brought forward	(2 693)	
earned income	(100)	(200)
Net requirement	6 025	9 302

The 1983 budget has been reviewed and all cost estimates have been reconsidered in relation to 1982 expenditures and the research program now anticipated for 1983. The budget is patterned according to ILRAD's current research programs and administrative structure. The two main research programs are not costed separately, but it is estimated that expenditures for the trypanosomiasis program are about 57% of the total.

Based on current estimates of operating costs, the 1983 budget has been revised so that core operations are somewhat below the bottom level approved by the CGIAR. Capital expenditures have been set at the top approved level. The net effect is a total at the bottom level.

The operating budget for 1983 is sufficient to support 46 senior manyears. The capital budget includes those items approved by the Board of Directors, plus the purchase of laboratory equipment and the construction of an incinerator, sheep- and goat-holding facilities and a house for the Director General. These last two projects have been shifted forward from 1984. Details are given in section 10.

Most of the laboratories will have more senior staff in 1983 than in 1982. About 8.0 additional manyears are expected in the research program and 10.0 manyears overall. As a result of staff increases, operating costs will be higher in 1983 for the Biochemistry, Immunology and Pathology Laboratories, while operating

costs for the other laboratories will be similar to 1982 levels. In the presentation of the core operating budget for 1983 (Table A-4), the staff of some laboratories are also aligned differently than they were in 1982, which affects the calculation of their costs. The overall cost increase is 17% above the 1982 level, including 8% due to price changes.

Costs for research support will increase substantially in 1983, primarily because the Director of Research position was activated at the beginning of the year. Costs associated with this post are included in the research support category, along with costs formerly attributed to collaborative research.

Costs for farm animal production will increase due to the project to introduce N'Dama cattle to the Kabete site by ova transplant procedures. This has added US $90 000 to the 1983 budget for the Large Animal Unit (Kabete farm). However, once an N'Dama breeding herd is established at ILRAD, further transplants will not be necessary so the costs for this unit should be reduced in 1984.

The most significant increase in expenditures in 1983 will be for Training and Conferences. This reflects the increased emphasis being given to training at ILRAD. Individual training programs and courses have been increased in 1983, and an international conference is being held.

A timely reporting system for expenditures was activated in 1981. Better financial control was achieved in 1982, along with more effective use of funds, and further gains in efficiency are anticipated in 1983. Administrative and general operating costs are expected to increase only slightly over the 1982 level.

6. 1984-85 BUDGET REQUEST

Table 3. 1983 CURRENT BUDGET AND CURRENT REQUESTS FOR 1984-85 (US$'000)

	1983 Current Estimate	1984 Current Request	1985 Current Request
Core operations	8 562	8 954	8 880
Contingency	170	170	170
Capital	770	727	500
Price change	-	868	1 805
Total	9 502	10 719	11 355
Less earned income	(200)	(200)	(200)
Net requirement	9 302	10 519	11 155

The 2-year budget for 1984-85 has been developed using baseline figures allocated to ILRAD by the CGIAR secretariat.

The current program of steady recruitment will lead eventually to a full complement of staff at the level authorized for ILRAD as appropriate for the laboratory facilities which have been constructed (51 senior positions). Present recruitment objectives call for six new staff members in 1983 and four in 1984.

The 1984 and 1985 budget requests presented here are the minimum amounts required for operation at full or nearly full staffing levels. Any new initiatives would be possible only at the expense of other activities.

The 1985 budget proposal is based on guidelines provided and represents a no-growth budget. Because it is difficult to develop an accurate request for 1985 with several factors remaining uncertain for 1983 and 1984, the request for 1985 is provisional and will be subject to modification.

7. SPECIAL PROGRAM AND BUDGET CONSIDERATIONS

The original budget for 1983 included funds for an addition to the administrative block. At present, 25% of ILRAD's total administrative office space is allocated to regional staff of the International Potato Centre (CIP), the International Maize and Wheat Improvement Centre (CIMMYT) and the International Board for Plant Genetic Resources (IBPGR). ILRAD has also made one three-bedroomed house available to ILCA for offices, and in 1981 ILCA constructed a prefabricated building on the ILRAD site to provide another 15 offices. At least one other centre in the CGIAR system has also expressed its intention to place a staff member at ILRAD.

Because Nairobi is an appropriate location for regional staff in East Africa, office space requirements for other CGIAR centres are likely to continue and even expand. ILRAD's own needs for administrative offices are also likely to increase. So far, it has been possible to meet the needs of other centres, but this may not be feasible in future. If existing office space becomes inadequate, no doubt ILRAD will have to put its own needs first.

Since this item is largely in support of other centres, it has not been listed in the capital budget request. To do so would mean a reduction of funds from ILRAD's operating budget in order to stay within the allocated budget base. However, the donors may wish to consider construction of additional office space at ILRAD as a broader need of the CGIAR system as a whole.

Meanwhile, ILRAD has developed a plan to remodel the original stores building into office space for regional scientists from other CGIAR centres. This space has become available because of efforts during the past 2 years to reduce ILRAD's inventory of stock. The conversion of this space will provide 11 offices and space for support staff at an estimated cost of US $15 000. This work will be carried out in 1983.

8. COMMENTS ON THE CORE PROGRAM FOR 1984 AND 1985

Table 4. ALLOCATION OF SCIENTIFIC MANYEARS TO PROJECT AREAS IN THEILERIOSIS AND TRYPANOSOMIASIS RESEARCH, 1984

	Manyears	% Program	% Total Research
Theileriosis Program			
1. Epidemiology	3.0	16.0	7.7
2. Sporozoite immunization	8.2	43.0	21.0
3. Schizont immunization	7.7	41.0	19.8
Total	18.9	100.0	48.5
Trypanosomiasis Program			
4. Epidemiology	3.1	15.0	7.9
5. Trypanosome antigenicity	10.6	53.0	27.2
6. Comparative immunology	6.4	32.0	16.4
Total	20.1	100.0	51.5

Research at ILRAD is organized in six multidisciplinary project areas, each with a project leader and defined objectives related to the goal of improved theileriosis and trypanosomiasis control. Table 4 gives an estimate of the scientific manyears which will be allocated to each area in 1984. Subject to the availability of core funds, four additional scientific manyears will be added in 1984, two in theileriosis research, one in trypanosomiasis research and one visiting scientist position for a consultant biometrician, allocated to research support.

THEILERIOSIS

1. Epidemiology

The objectives of ILRAD's theileriosis epidemiology project are to increase knowledge of the epidemiology of theileriosis in Africa and to introduce and evaluate immunological and chemoprophylactic control methods in endemic areas in collaboration with national and international programs.

Strains of T p parva isolated from different areas of Kenya and neighbouring countries have been characterized at ILRAD and their potential value for inclusion in vaccination trials is being determined. Pilot immunization trials, based on infection with live parasites and treatment, have been carried out in one area of Kenya and will be extended in Kenya and to other affected countries. One additional scientific position will be allocated to this project in 1984. This is a long-term project.

2. Immunization with non-viable sporozoite material

The objective of ILRAD's Theileria sporozoite project is to identify, characterize and produce antigens from the sporozoite form of T p parva which will induce protective immunity.

Antigenic components of T p parva have been detected which give rise to protective antibody responses. These will be characterized and the regions of the molecules responsible for protection determined. The next step will be to investigate the feasibility of producing the relevant regions in vitro by recombinant DNA technology or other synthetic procedures. Products of these systems will then be tested for their ability to induce protection in vivo when combined with a suitable adjuvant. The role of tick salivary proteins in establishing Theileria infections will also be investigated. Significant results should be obtained within 5 years.

3. Immunization wih material from schizont-infected cells

ILRAD's Theileria schizont project explores the possibility of inducing protective immunity against T p parva using antigens associated with the schizont form of the parasite.

ILRAD scientists are delineating the nature of the bovine lymphoid cells which become infected and transformed by the schizont form of T p parva. Scientists are also investigating the changes induced in these cells which allow them to be recognized and destroyed by other cells of the bovine immune system. Antigens identified from these infected cells are being characterized, and their potential is being assessed for use in inducing immunity. This is a long-term project requiring definition of the components of the bovine immune system.

Work in this area is being strengthened by an additional visiting scientist and research technician, partially supported by ILRAD core funds and partially by funds from the British Overseas Development Administration (ODA). A further scientific position will be added from ILRAD core resources in 1984.

TRYPANOSOMIASIS

4. Epidemiology

The objectives of the trypanosomiasis epidemiology project are to identify the factors which contribute to tsetste/trypanosomiasis challenge in different climatic regions of Africa, to monitor - and if possible improve - the use of available drugs, and to assess the

potential role of trypanotolerant livestock in improving livestock productivity in tsetse-infested areas.

The results of a pilot study at the Kenya coast are being analysed to assess the role of acquired immunity to local trypanosome populations and to determine the role of chemotherapy in the acquisition of immunity. Collaborative studies will continue with ILCA and national organizations on the nature of trypanotolerance and the productivity of domestic livestock under different levels of management and degrees of trypanosomiasis risk. ILRAD and ILCA, together with ODA and FAO, will participate in a large-scale special project in The Gambia on the use of trypanotolerant N'Dama cattle to increase livestock productivity. Support for ILRAD's participation will be provided by the EEC, including funding for two additional scientific positions. This is a long-term project.

5. Trypanosome antigenicity and biochemistry

Trypanosome antigenicity and biochemistry studies at ILRAD are designed to obtain knowledge of the properties of the major pathogenic trypanosome species, especially relating to their antigenicity, pathogenicity and biochemistry. Knowledge is sought which can be used for the prevention or improved control of African trypanosomiasis.

Studies will continue on the role of the variable surface antigens in the induction of protective immunity. Antigens are being analysed from the metacyclic and bloodstream stages of parasite development, focusing particularly on T congolense and T vivax. The structure of these antigens will be determined, along with the way they are synthesized and their genetic basis. Work will also continue on the identification and characterization of non-variable antigens which stimulate immune responses in livestock, focusing on the potential use of these antigens for diagnosis and the stimulation of protective immunity.

The metabolic pathways related to parasite survival and differentiation will be defined, and the development of improved methods to propagate trypanosomes in vitro will continue. The effectiveness of available and newly derived trypanocidal compounds will be tested and their mode of action studied in vitro and in vivo. An additional parasitology position will be allocated to this project in 1984. This is a long-term project.

6. Comparitive immunology including genetic resistance to trypanosomiasis

The objective of ILRAD's trypanosomiasis immunology project is to identify the immunological and non-immunological mechanisms which contribute to innate and acquired resistance to trypanosomiasis.

Studies are carried out in laboratory animals, domestic livestock and indigenous East African wildlife species. Work on resistant wild animals is being carried out in collaboration with the Kenya Government's Veterinary Research Laboratories, supported by The Netherlands. The N'Dama cattle which have been imported into

Kenya by ova transplant will be used for research at Kabete, and N'Dama will be available in The Gambia for limited experimental work, with EEC support. This is a long-term project.

9. SPECIAL PROJECTS

WILDLIFE DISEASE RESEARCH TO CONTROL DISEASES OF ECONOMIC IMPORTANCE

A 3-year wildlife disease research project was initiated in 1981, involving one senior scientist. Funding is provided by the Government of The Netherlands, and research is conducted at the Kenya Veterinary Research Laboratories at Kabete. Funds for the project are administered through ILRAD.

Table 5. WILDLIFE DISEASE RESEARCH PROJECT BUDGET (US $)

	Actual 1981	Actual 1982	Proposed 1983
Salaries	59 800	79 700	82 000
Supplies	12 700	28 300	38 000
Travel	3 300	9 600	3 200
Capital	31 300	4 2000	
Training	3 700	15 200	5 800
Contingency	12 200	14 600	12 000
Total	123 000	151 600	141 000

STUDIES ON THE INFECTION RATES AND TRANSMISSION CHARACTERISTICS OF PATHOGENIC TRYPANOSOMES IN LABORATORY-REARED TSETSE

A research project, 'Studies on the Infection Rates and Transmission Characteristics of Pathogenic Trypanosomes in Laboratory-Reared Tsetse as Part of the SIT Program', was initiated in December 1979 and is scheduled to continue through October 1983. As partial support for this project, the International Atomic Energy Agency (IAEA) provides US $5 500 a year as a research contract. This project is part of a larger IAEA program aimed at tsetse eradication in Nigeria.

N'DAMA PRODUCTION RESEARCH

A proposal for funding a special research project on trypanotolerant N'Dama cattle has been submitted to the EEC jointly by ILRAD and ILCA. This project will be the research component of a larger regional project developed by the Government of The Gambia and

funded by several donors. The goal is to increase the production and export of N'Dama cattle.

This project.would allow ILRAD to carry out substantial research on the mechanisms of trypanotolerance. Together with research carried out by ILCA, ILRAD's research findings should make it possible to evaluate the potential productivity of the N'Dama breed in areas of defined tsetse challenge.

The proposal under consideration by the EEC would provide funds for ILRAD to hire two scientists who would be stationed in The Gambia. The project would be carried out over a 3-year period, with an annual budget of approximately US $750 000 for the combined ILRAD/ILCA component. The original plans called for the project to start in mid-1983. However, notification of funding is still awaited.

SUPPORT FOR TRYPANOSOMIASIS RESEARCH

One scientist is seconded to ILRAD by the Government of The Netherlands to study the skin responses of ruminants when bitten by tsetse infected with trypanosomiasis. All funds for personal services and research costs are paid to ILRAD. The project started on 15 May 1982 and is to end on 14 May 1984. The budget for this project is given in Table 6.

Table 6. TRYPANOSOMIASIS RESEARCH SUPPORT BUDGET (US $)

	1982 7.5 Months	1983 12 Months	1984 4.5 Months
Personal Services	31 500	50 000	23 000
Travel	500	2 000	1 000
Supplies	2 000	12 000	3 000
Total	34 000	64 000	27 000

10. CAPITAL REQUIREMENTS

The major capital development project in 1982 was the construction of a third group of staff houses at ILRAD. These eight houses were completed on time and within the budget allocated. Six of them were occupied immediately.

Tables A-1 to A-3 list actual and proposed capital expenditures. In 1982, expenditures exceeded expectations by only US$ 16 000. There was an over-expenditure for capital improvements on the ranch at Kapiti and other over-expenditures for small projects and improvements at the ILRAD site in Kabete. In addition, payments were made to settle two long-standing disputes with contractors, one concerning the second group of staff houses and one concerning the third cattle holding unit at the ILRAD site. These were partly balanced by under-expenditures for construction of the third group of staff houses and for an addition to the tick unit.

Capital funds of US $450 000 were approved for 1983. However, when the 1983 budget was revised it became apparent that additional capital could be spent without going over the approved bottom level. As a result, certain items which had been projected for 1984 were moved forward to 1983. This included US $200 000 for a house for the Director General, US $50 000 for a sheep- and goat-holding unit, and US $50 000 for additional laboratory equipment.

The shift of the two construction projects to 1983 means that no substantial construction projects are planned for 1984 or 1985. Any construction undertaken during this period will probably involve modifications of existing facilities as research requirements change.

11. ASSUMPTIONS ON PRICE CHANGES

The consumer price index for Kenya increased by nearly 20% in 1982, and in 1983 the increase will almost certainly be larger. However, the Kenya Government approved two devaluations of the Kenya shilling in 1982, and it is likely that the shilling will be devalued again if this is dictated by economic factors. It is not likely, however, that a future devaluation will be sufficient to compensate fully for the rate of inflation.

Considering all these factors, ILRAD has estimated annual price changes between the compound value of 8% for Europe and North America and the net value for Kenya, which will probably be at least 10%. At this time, there is no reason to suggest a value different from that provided by the CGIAR Secretariat.

12. PRESENTATION OF THE BUDGET AT THE BOTTOM OF THE BRACKET RECOMMENDED BY TAC

To limit ILRAD's proposed 1984 budget by US $814 000, in line with the bottom of the bracket recommended by TAC, reductions would be made as shown in Table A-10.

The first response to a budget reduction would be to postpone or seek outside funding for a scientific conference planned for 1984. The second response would to reduce expenditures for training, operating and laboratory equipment.

As a last resort, reductions would have to be made in senior scientific staff positions. Whenever possible, this would be done by allowing any vacant positions to remain open. It has been estimated that each position left unfilled for one year results in a budget reduction of US $200 000, but this could be an overestimate since support costs do not usually fluctuate immediately in response to the addition or loss of a scientific position.

Since 1981, ILRAD management has been working steadily to recruit a full complement of scientific staff in order to utilize to the best advantage the equipment and facilities available at ILRAD. While a conference or the purchase of equipment could be delayed without major disruption, a reduction in staff would seriously constrain efforts to attain ILRAD's research goals.

APPENDIX: TABLES

Table A-1. SUMMARY OF CAPITAL BUDGET, 1982-86 (US $'000)

	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed	1986 Projected
CONSTRUCTION					
Staff Housing	630	200			
Addition to Tick Unit	45				
Development of Kapiti Plains Ranch	130	70	20	20	
Sheep & Goat Holding Unit		50			
Small Projects & Site Improvement	245	100	50	130	150
TOTAL	1050	420	70	150	150
EQUIPMENT					
Laboratory & Autoclave (Small Animal Unit)	257	150	360	192	220
Farm	8	50	40	30	50
Office	13	20	15	10	10
Vehicles	20	30	30	30	30
Food & Housing	1	5	5	5	5
Engineering	17	55	10	5	10
Training Laboratory			100		
TOTAL	316	310	560	272	325
ADDITIONAL WORKING CAPITAL		40	97	78	80
TOTAL CAPITAL	1366	770	727	500	555

Table A-2. DETAILS OF CAPITAL BUDGET: CONSTRUCTION, 1982-86 (US $'000)

	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed	1986 Projected
SMALL PROJECTS & SITE IMPROVEMENT					
Vehicle Park & Workshops	102				
Fire Control		15			
Farm Roads	47	15			
Reservoir Washout	15				
Radioisotope Laboratory Improvements	2	10			
Site Improvements		7			
Grounds Staff Facility		5			
Cold Room - Laboratory 4	14				
Incinerator		3			
Water Storage Tank		4			
Small Modifications & Unidentified	65	41	40	120	130
Contingency			10	10	20
TOTAL	245	100	50	130	150

Table A-3. DETAILS OF CAPITAL BUDGET: EQUIPMENT, 1982-86 (US $'000)

	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed	1986 Projected
LABORATORY EQUIPMENT					
Liquid Chromatograph (HPLC)	42				
Rotor-Beckman (SW 41 Ti)	10				
Rotor-Beckman (SW 50.1)	13				
Incubator (CO_2)		6			
Liquid Nitrogen Tanks	15				
Freeze Driers (2)	13				
Centrifuge (RT 6000)	12				
Combi Cold Rac (LKB)	7				
Centrifuge (RC-3B Sorvall)	19				
Cytocentrifuge	3				
Radiation Monitor	7				
Densitometer	7				
Osmometer	5				
Microcomputer	19				
Programmable Freezer	8				
Laminar Flow Hoods	2	4			
Fluorimeter Attachment		5			
Frozen Thin Sectioning System		8			
Micro ELISA Reader		15			
Fluorescence Activated Cell Sorter Equipment	23	45			
Autoclave			160		
Other	52	67	200	192	220
TOTAL	257	150	360	192	220
FARM EQUIPMENT - Small Equipment	8	50	40	30	50

OFFICE EQUIPMENT					
Word Processing Equipment	10	5			
Unidentified	3	15	15	10	10
TOTAL	13	20	15	10	10
VEHICLES - Work & Staff Vehicles	20	30	30	30	30
FOOD & HOUSING - Catering & Laundry Equipment	1	5	5	5	5
ENGINEERING					
Maintenance Equipment	17	5	10	5	10
Incinerator (Radioactive Waste)		50			
TOTAL	17	55	10	5	10
TRAINING LABORATORY EQUIPMENT					
Microscopes (8)			40		
Spectrophotometers (4)			12		
LKB Fraction Collector & Accessories			4		
Balances (4)			6		
Centrifuge (Refrigerated)			8		
Freezer			4		
Micro ELISA Readers (2)			7		
Other			19		
TOTAL			100		
TOTAL ALL EQUIPMENT	316	310	560	272	325

Table A-4. SUMMARY OF COSTS BY PROGRAM AND ACTIVITY, 1981-85 (US $'000)

	1981 Actual		1982 Actual		1983 Final Revised		1984 Proposed		1985 Proposed	
	M-Y	Cost	M-Y	Cost	M-Y	Cost	M-Y	Cost	M-Y	Cost
			(Botton Bracket)							
RESEARCH										
Parasitology-Tryps	5.0	469	5.1	446	5.0	450	5.3	453	6.0	472
Biochemistry	4.7	554	5.7	483	9.2	638	7.8	648	8.0	649
Cell Biology	2.7	344	3.0	382	3.4	394	5.3	418	6.0	418
Immunobiology	4.9	455	3.1	285	5.6	455	7.0	475	7.0	476
Parasitology-ECF	4.8	493	5.7	449	6.0	533	6.3	580	6.0	578
Pathology	4.2	483	4.1	448	5.6	581	5.3	633	4.4	630
Tsetse Lab	1.3	312	1.0	301	1.0	306	1.0	308	1.0	308
Tick Lab	1.0	76	0.7	93	1.0	116	1.0	119	1.0	120
Electron Microscopy	1.0	117	1.3	129	1.0	133	1.0	135	1.0	135
Collaborative Research		64		142						
TOTAL	29.6	3367	29.7	3158	37.8	3606	40.0	3769	40.4	3786
RESEARCH SUPPORT										
Research Director					1.0	310	1.0	293	1.0	280
Kabete Farm	0.6	458	1.0	573	1.0	644	1.0	573	1.0	580
Small Animal Unit		128		131		135		137		136
Veterinary Services	0.9	84	0.2	63	1.0	79	1.0	79	1.0	82
Central Core Lab		303		400		351		354		342
TOTAL	1.5	973	1.2	1167	3.0	1519	3.0	1436	3.0	1420
TOTAL RESEARCH	31.1	4340	30.9	4325	40.8	5125	43.0	5205	43.4	5206
TRAINING & CONFERENCES	1.0	301	1.0	526	1.0	756	1.0	980	1.0	900

LIBRARY & INFORMATION		166	1.0	267	1.0	335	1.0	348	1.0	348
ADMINISTRATION										
Board of Directors		81		70		92		92		93
Director General's Office	1.8	416	2.0	319	2.0	326	2.0	362	2.0	363
Finance	1.0	509	1.0	379	1.0	395	1.0	427	1.0	426
Purchasing		223		257		276		283		284
Personnel		54		73		85		87		89
TOTAL	2.8	1283	3.0	1098	3.0	1174	3.0	1251	3.0	1255
GENERAL OPERATIONS										
Engineering	1.0	536	1.0	523	1.0	622	1.0	643	1.0	653
Transport		206		185		245		222		228
Services		313		275		162		184		186
Food & Housing	1.0	233	1.0	33	1.0	99	1.0	72	1.0	56
Stores		67		54		44		49		48
TOTAL	2.0	1355	2.0	1070	2.0	1172	2.0	1170	2.0	1171
OTHER										
Contingency				166		170		170		170
Price Change								868		1805
TOTAL				166		170		1038		1975
TOTAL CORE	36.9[a]	7445	37.9[b]	7452	47.8[c]	8732	50.0	9992	50.4	10855
SPECIAL PROJECTS		123		141		205		27		

[a]Includes 3.7 M-Y from other funds. [b]Includes 3.4 M-Y from other funds. [c]Includes 3.5 M-Y from other funds.

Table A-5. SUMMARY OF COSTS BY OBJECT OF EXPENDITURE, 1981-85 (US $'000)

	1981 Actual	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed
Personnel Costs	4129	4451	5198	5506	5524
Supplies	2106	1547	1944	1945	1950
Services	749	778	695	710	646
Equipment Replacement	151	232	245	250	250
Travel	310	278	480	550	510
Other/Contingency		166	170	1031	1975
TOTAL	7445	7452	8732	9992	10855

Table A-6. SUMMARY OF APPLICATIONS AND SOURCES OF FUNDS: APPLICATIONS, 1981-85 (US $'000)

	1981 Actual	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed
APPLICATION OF FUNDS					
Core Operations	7445	7452	8732	9992	10855
Capital	2340	1366	730	630	422
Special Projects	123	81	205	27	
UNEXPENDED BALANCES					
Unrestricted Core	2648	881			
Capital	45	181			
Working Funds	709	709	749	846	924
Special Projects	(71)	(21)			
TOTAL APPLICATIONS	13239	10649	10416	11495	12201
MEMO					
Total Core Operating Funds Required	7445	7452	8732	9992	10855
Less Unexpended Balance from Previous Year	(2131)	(2648)	(881)		
Less Earned Income Applied	(184)	(100)	(200)	(200)	(200)
Recoverable from Special Project			(21)		
NET CORE OPERATING FUNDS REQUIRED	5130	4704	7630	9792	10655
Total Capital Funds Required	3049	2075	1479	1476	1346
Less Unexpended Balance from Previous Year	(637)	(45)	(181)		
Less Previous Working Funds	(709)	(709)	(709)	(749)	(846)
NET CAPITAL FUNDS REQUIRED	1703	1321	589	727	500
TOTAL FUNDS REQUIRED FROM CGIAR	6833	6025	8219	10519	11155

Table A-7. SUMMARY OF APPLICATIONS AND SOURCES OF FUNDS: SOURCES, 1981-85 (US '$000)

	1981 Actual	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed
CORE OPERATING FUNDS					
UNRESTRICTED					
IDA	1910	280	800		
West Germany	770	717	603		
United Kingdom (ODA)	540	559	548		
Canada (CIDA)	505	545	615		
Switzerland	412	391	383		
Norway	380	325	261		
Belgium	258	186	151		
Rockefeller Foundation	100				
Netherlands	230	295	313		
Australia	265	286	285		
Sweden	185	200	186		
Ireland	100				
Italy			136		
Saudi Arabia			300		
IAEA		5	5		
Unidentified			154	10519	11155
UNEXPENDED BALANCES					
From Previous Year	2131	2648	881		
Earned Income Applied in Year	184	100	200	200	200
TOTAL UNRESTRICTED	7970	6537	5821	10719	11355

RESTRICTED					
USAID	3100	2400	2500		
UNDP	694	799	576		
Belgium	77	99			
Italy			350		
France			53		
TOTAL RESTRICTED	3871	3298	3479		
TOTAL UNRESTRICTED/RESTRICTED	11841	9835	9300	10719	11355
Transfer to Capital Funds	(1749)	(1321)	(589)	(727)	(500)
TOTAL OPERATING FUNDS	10092	8514	8711	9992	10855
CAPITAL					
Transferred from Core	1749	1321	589	727	500
Unexpended Balance from Previous Year	637	45	181		
Balance of Working Funds from Previous Year	709	709	709	749	846
TOTAL CAPITAL FUNDS	3095	2075	1479	1476	1346
SPECIAL PROJECT - Netherlands	52	60	226	27	
TOTAL FUNDS	13239	10649	10416	11495	12201

Table A-8. SUMMARY FINANCIAL DATA, 1981-85 (US $'000)

	1981 Actual	1982 Actual	1983 Final Revised	1984 Proposed	1985 Proposed
CURRENT ASSETS					
Cash	3505	2205	749	846	924
Receivables	479	581	500	500	500
Inventories	406	227	200	200	200
Prepaid Expenses	110	147	150	150	150
Current Account with Subsidiary (Kapiti)	191	184	200	200	200
TOTAL CURRENT ASSETS	4692	3344	1799	1896	1974
FIXED ASSETS					
Operating Equipment	352	626	686	731	771
Research Equipment	4150	4485	4635	4885	5077
Vehicles	330	282	312	312	342
Furnishings & Office Equipment	507	467	487	487	497
Buildings & Land	8765	9390	9740	9740	9890
Investment in Kapiti Ranch	1521	1651	1721	1721	1721
Other Fixed Assets	292	165	185	185	185
TOTAL FIXED ASSETS	15917	17066	17766	18061	18483
TOTAL ASSETS	20609	20410	19565	19957	20457

LIABILITIES					
Accounts Payable	305	695	750	750	750
Other Liabilities	601	253	300	300	300
TOTAL LIABILITIES	906	948	1050	1050	1050
GRANTS RECEIVED IN ADVANCE	384	623			
CAPITAL GRANTS					
Fully Expended	15917	17066	17766	18061	18483
Unexpended	709	709	749	846	924
TOTAL CAPITAL GRANTS	16626	17775	18515	18907	19407
UNEXPENDED CORE OPERATING GRANTS	2693	1064			
TOTAL LIABILITIES & GRANTS	20609	20410	19565	19957	20457

Table A-9. POSITIONS AND MANYEARS, 1982-85

	SCIENTIFIC AND SENIOR ADMINISTRATIVE STAFF								SUPERVISORY STAFF								SUPPORT STAFF							
	Positions				Manyears				Positions				Manyears				Positions				Manyears			
	82	83	84	85	82	83	84	85	82	83	84	85	82	83	84	85	82	83	84	85	82	83	84	85
Research	41	41	41	41	29.7	37.8	40.0	40.4	20	20	20	21	17.9	19.0	19.0	20.0	68	75	75	75	64	70	70	70
Research Support	2	3	3	3	1.2	3.0	3.0	3.0	4	4	4	4	4.0	4.0	4.0	4.0	87	85	85	85	81	80	81	81
Training & Conferences	1	1	1	1	1.0	1.0	1.0	1.0	1	1	1	1	1.0	1.0	1.0	1.0								
Library & Information	1	1	1	1	1.0	1.0	1.0	1.0	1	1	1	1	2.0	1.0	1.0	1.0	7	7	7	7	6	7	7	7
Administration	4	3	3	3	3.0	3.0	3.0	3.0	10	10	10	10	8.0	9.6	10.0	10.0	20	22	22	22	18	20	21	21
General Operations	2	2	2	2	2.0	2.0	2.0	2.0	3	3	3	3	3.0	3.0	3.0	3.0	120	129	130	132	116	123	128	130
TOTAL	51	51	51	51	37.9	47.8	50.0	50.4	39	39	39	40	35.9	37.6	38.0	39.0	302	318	319	321	285	300	307	309
OF THESE:																								
NON-ILRAD FUNDED					3.4	3.5																		

SUMMARY

Year	Positions	Manyears	% Positions Filled
1982	392	359	92
1983	408	385	94
1984	409	395	97
1985	412	398	97

Table A-10. PLAN TO REDUCE EXPENDITURE TO BOTTOM OF BRACKET (US $'000)

	Senior Manyears	OPERATIONS In 1983	Price Provision	In 1984	Adjustment of Working Capital	CAPITAL Capital	Total	TOTAL REQUIREMENTS Gross	Net
1984 REQUEST	50	9124	868	9992	97	630	727	10719	10519
DEDUCTIONS									
1. Postpone one international conference		(73)	(7)	(80)	(6)		(6)	(86)	(86)
2. Postpone expenditure for training laboratory equipment						(100)	(100)	(100)	(100)
3. Reduce expenditure for operating equipment						(45)	(45)	(45)	(45)
4. Reduce expenditure for laboratory equipment						(150)	(150)	(150)	(150)
5. Leave two positions unfilled	(2)	(350)	(50)	(400)	(33)		(33)	(433)	(433)
SUBTOTAL	(2)	(423)	(57)	(480)	(39)	(295)	(334)	(814)	(814)
BOTTOM OF BRACKET	48	8701	811	9512	58	335	393	9905	9705