OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

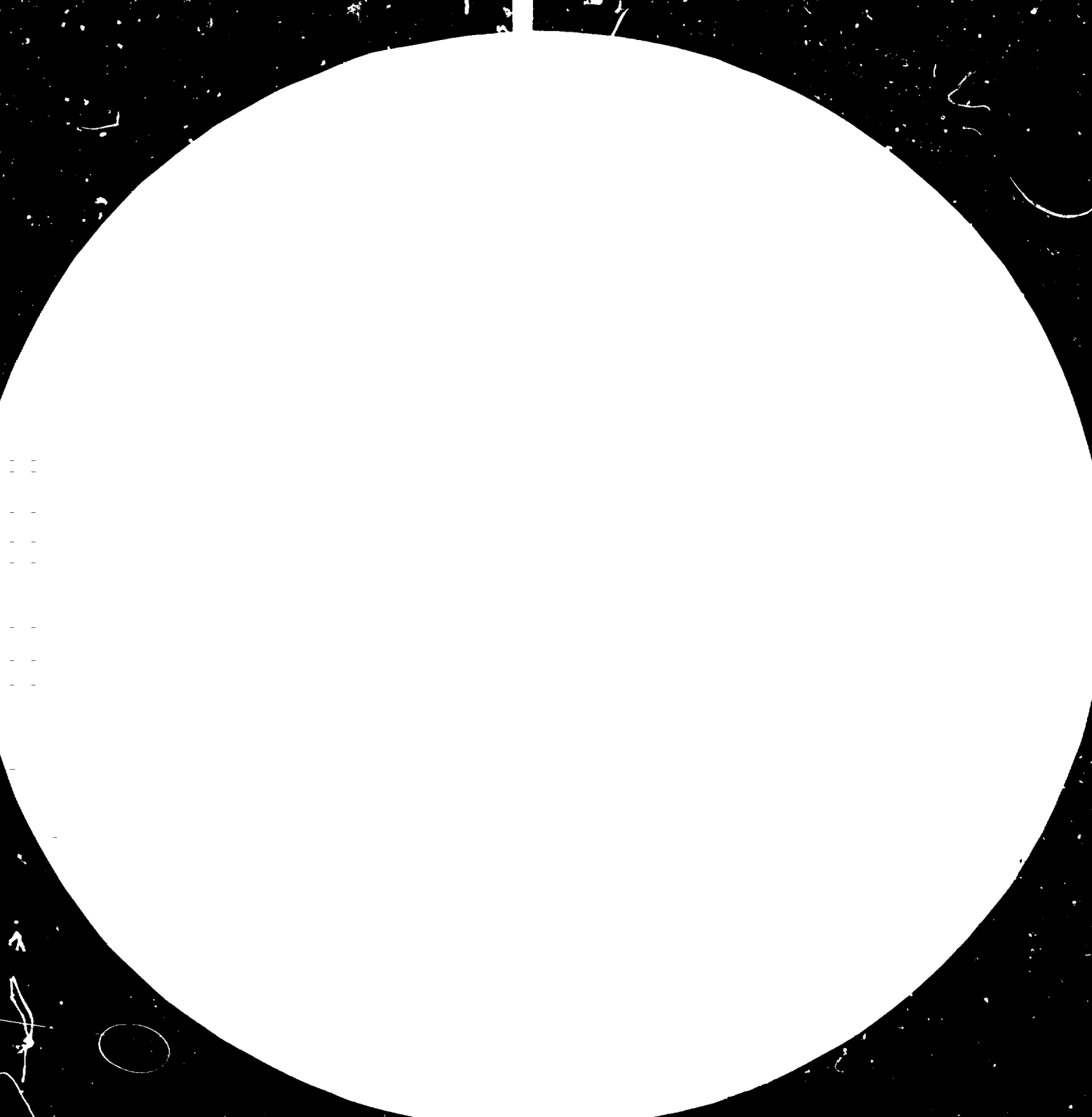
FAIR USE POLICY

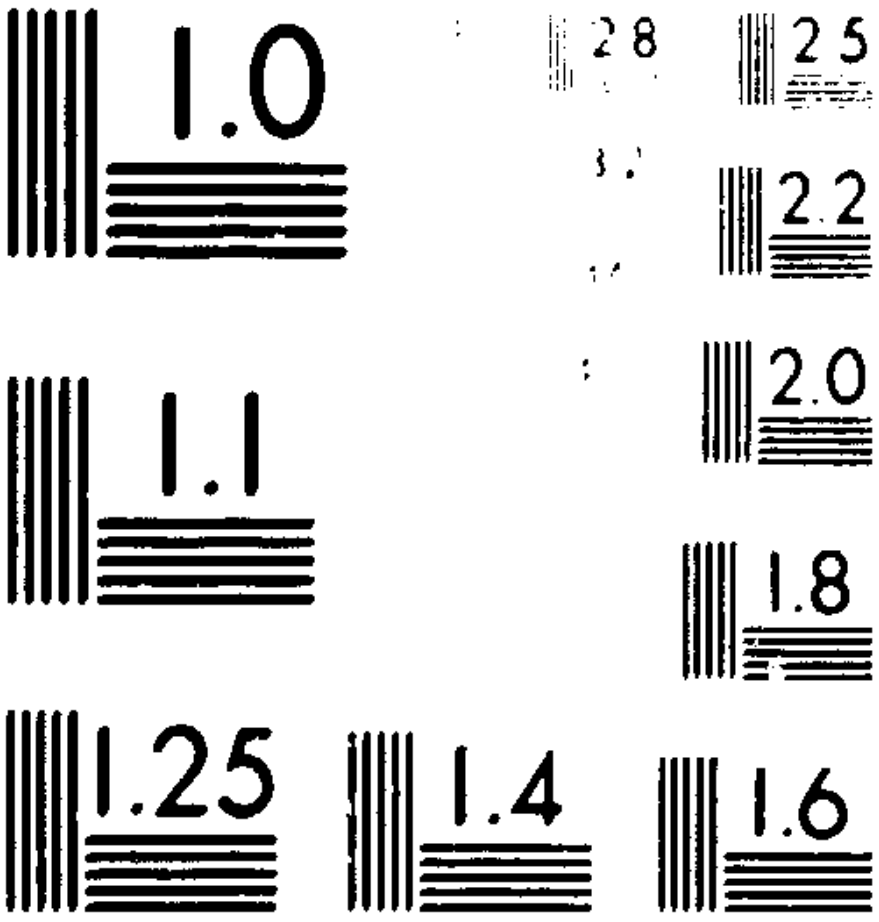
CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org




1.0
2.8
2.5
2.2
1.1
2.0
1.8
1.25
1.4
1.6

11732

Distr.
LIMITED

ID/WG.382/2/Add.4
20 September 1982

ORIGINAL: ENGLISH

United Nations Industrial Development Organization

High-level Meeting on the Establishment of the International Centre for Genetic Engineering and Biotechnology

Belgrade, Yugoslavia, 13-17 December 1982

APPLICATION OF GENETIC ENGINEERING AND BIOTECHNOLOGY FOR THE PRODUCTION OF IMPROVED HUMAN AND ANIMAL VACCINES WITH PARTICULAR REFERENCE TO TROPICAL DISEASES*

prepared by

Ahmad Bukhari**

and

Ulf Pettersson***

00[illegible]

* The views expressed in this paper are those of the authors and do not necessarily reflect the views of UNIDO. This document has been reproduced without formal editing.

** Senior scientist, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

*** Professor, Department of Medical Genetics, Biomedical Center, University of Uppsala, Box 589, 751 23 Uppsala, Sweden

V.82-30507

Contents

A. BACKGROUND AND JUSTIFICATION

Advances in our knowledge of the processes of life at the molecular level have brought us to the threshold of a new technology and a new industry. Many intricate biological problems that seemed so distant to a scientist a few years ago can now be studied and solved with the new techniques. Much of the work which is being done now will lead in a few years to practical applications whose impact in human and economic terms may be immense. One of the earliest applications of the new technologies will be to solve the problems of infectious diseases, still prevalent in the world. Tropical diseases affect hundreds of millions of people. Since molecular biologists are now in a position to attack these problems with reasonable chances of success it has become incumbent upon us to try to solve these, and alleviate the miseries of persons in as short a time as possible. So far, all the modern technological work on the diseases important in developing countries, has been done in the Western countries. For example, nearly a third of the world's population lives in malaria risk areas, almost all of which are in the developing countries. Yet the malaria vaccine research by genetic engineering techniques is located entirely in the United States and western Europe. One of the main reasons for this is the poor state of scientific and technological research especially in the new biotechnologies in the developing countries. Some of the research done in the advanced countries may be tied to commercial interests and it is not clear how the problems of production and marketing will be resolved.

It is thus necessary that a programme is initiated on the tropical diseases at the International Centre for Genetic Engineering and Biotechnology (ICGEB). Since not all the problems can be solved at the same time and since there are constraints on resources, we have to ask

(a) what problems should we try to solve first? and

(b) how should we try to solve them?

This work programme discusses some general answers to these questions and proposes some specific projects to be carried out and started immediately.

A discussion of pharmaceuticals and drugs in the coming years should cover not only human diseases but also animal diseases and perhaps plant diseases of economic importance. However, a discussion of all of these important diseases even on a cursory level is not possible since the number of diseases to be covered would be vast. Therefore, the main emphasis here is on human diseases. The general approach, and even specific approaches to the problems of animal diseases, would be the same as in human diseases.

Human diseases can be roughly divided into three categories:

(a) infectious diseases;
(b) genetic disorders; and
(c) general pathological disorders of the human body.

Many infectious diseases are still common in the developing countries and a vast number of persons are infected in the tropics every year. Genetic diseases, such as those affecting blood constituents and hormones, are found all over the world, although some may be prevalent in particular populations and regions (for example, sickle cell anemia is prevalent in African populations and beta-thalassemia in Mediterranean populations). General diseases of humans know no boundaries; cancer, heart ailments, respiratory diseases, degenerative diseases, etc., are major problems in the industrially advanced countries. However, it should be noted that with the industrial development and increased uses of chemicals, the incidences of these diseases in the developing countries are increasing at a rapid rate. The industrially advanced countries are spending a great deal of effort and money to understand the mechanisms by which many of these diseases are caused. These studies are long-term programmes of development with very generally defined goals. The problem is simpler in the case of tropical diseases. We know the causative agents of these diseases. Thus, if we can prevent the infectious agent from entering the human body; or if we can block its growth once it enters

the human body; or if we can kill the organism once the disease has set in, then we can eliminate the problem of a particular disease. It is because of this clear cut goal (to combat the infectious organism either by vaccine or by drugs) and real possibility of achieving such a goal that the work programme on tropical diseases promises to be a worthwhile and productive endeavour.

Important Infectious Diseases

Infectious diseases can be divided into three classes:

(a) those caused by parasites;
(b) those caused by bacteria; and
(c) those caused by viruses.

Table 1 and 2 list some of the common examples of such diseases. Some bacterial diseases are endemic in the developing countries. Parasitic diseases caused by worms or protozoa have a very high incidence rate and affect a very large number of persons. There are about one hundred and fifty million cases of malaria annually and in many countries the number of cases is growing. In Africa, more than a million children continue to die annually because of the disease. An estimated ten million persons are infected in South America by trypanosomes causing Chaga's disease. This disease causes congestive heart failure and other debilitating effects and there is no effective treatment for it. Other trypanosomes causing sleeping sickness in humans produce ten thousand new cases each year in Africa, but the disease constantly threatens to reach epidemic proportions as occurred at the beginning of the century. If untreated the disease is inevitably fatal due to invasion and destruction of the central nervous system. Furthermore, animal trypanosomiasis is a serious obstacle to human welfare because of the severe agricultural and economic problems it causes. Over three million cattle die each year from various forms of the disease and the rearing of domestic cattle, sheep and goats is

impossible in 10,000,000 square kilometers in Africa. Even these numbers seem small when one examines the incidence of diseases caused by worms of various sorts. Table 1 lists some of the very common diseases affecting persons.Very important diseases which could become targets of research at the ICGEB are malaria, schistosomiasis, filariasis, infantile diarrhoea, leprosy and trypanosomiasis.

Techniques Involved in Attacking the Problems

The much talked about potential of new technologies in helping us to make new vaccines and antibodies has come about because of two exceedingly important biological inventions.

Construction of recombinant DNA

The understanding of the structure and functioning of genetic information coded in DNA within the past decade has led to the advent of genetic engineering - the ability to manipulate DNA at will. DNA from one organism can be recombined with the DNA of another organism in test tubes, making recombinant DNA. The recombinant DNA can then be added to a living organism. In his way, genes from one bacterium can be added to another bacterium combining the useful properties of both bacteria. Similarly, genes from a plant or an animal can be transferred to a bacterium in such a manner that they will replicate as the bacterial genes do. Since bacteria multiply rapidly - the generation time can be as short as twenty minutes - and can be grown easily in the laboratory, it is possible to obtain large quantities of the genes transferred to these bacteria. Furthermore, the genes can be made to express themselves in these bacteria, providing easy access to important gene products which otherwise may be very difficult to obtain in large amounts. It may also be possible to transfer desirable genes to plants and to animals.

Cell technology

In the past twenty years there has been a steady progress in cell technology, which can be defined as manipulation of whole cells. Cell technology has several different aspects; one is changing the internal contents of the cell - implanting new genes into the cells or adding proteins and other substances to study their properties. The foreign material can be injected precisely into the cytoplasm, or into the nucleus through microinjection techniques using ultrafine needles. Techniques are also being developed to deliver the material inside the cell by enclosing it in microscopically small bags or capsules called 'liposomes' made up of fatty substances that easily merge with the membranes of the cells. Another aspect of cell technology is the growing of multicellular tissues from a single cell. This has been accomplished so far with plants, in which case a whole plant can be grown from a single cell. This, of course, has not been possible in animals. Generally, in animal systems, only embryonic cells are amenable to manipulations. In frogs, embryonic cell nuclei can be transplanted to eggs, whose nuclei have been removed, to form viable embryos. It has been possible to produce identical mice by similar procedures.

A strikingly successful form of cell technology is fusing two cells to make hybrid cells. Thus, if a cell making an important product cannot be grown readily, then it can be fused with another cell that does grow under laboratory conditions; a hybrid may be obtained that makes the required product. This principle has led to a breakthrough in the manufacturing of antibodies, which are substances made by animals to combat invasion of viruses, bacteria and other proteinaceous materials. Antibodies are normally obtained from the blood of animals. However, now a tumour cell with a potential for making antibodies can be fused with a cell that is actually making an important antibody. The hybrid cell then continues to multiply and gives rise to a clone of cells that grow easily like tumour cells, but produce the required antibody. This technique of making hybridomas to produce monoclonal antibodies is expected to have far-reaching consequences in medicine.

In general, it is simple to fuse any two cells by using agents such as viruses and chemicals that intermingle the membranes of the two cells. However, only the hybrid made from cells of a single species may remain properly functional; the hybrids made from cells obtained from two different species are not stable. This follows a fundamental rule of biology, that individuals of one species do not mate with the individuals of other species. This interspecies barrier keeps the infrastructure of our biological world stable. However, since cells of two species can be fused, it may be possible to derive functional hybrids in a limited sense. Some properties of one cell may be transferred to another. This is particularly important in plants since whole plants can be grown from single cells. Thus, cells of different plants may be fused to derive a plant with useful properties.

These techniques allow us to study in detail the genes and the gene products involved in pathogenesis. We can readily identify those antigens of a pathogenic organism that bring about an immune response in humans or animals. The genes for these antigens can then be cloned by recombinant DNA techniques into suitable host cells that can be propagated easily. The antigen can then be purified from these new host cells and antibodies obtained against them by the hybridoma techniques.

The Potential Use of Recombinant DNA Technology for Production of Human and Animal Vaccines

Techniques for production of efficient vaccines have been known for more than a century and it is today a well known fact that smallpox has been eradicated from the earth due to a successful vaccination programme. Also the poliomyelitis vaccination programme has been of tremendous importance for mankind. The classical methods for vaccine production are, however, in many respects combersome and expensive since the production of animal virus vaccines requires large-scale growth of tissue culture cells. Some microorganisms which are of great medical significance are, however, difficult to propagate under

laboratory conditions. It is anticipated that genetic engineering could circumvent these shortcomings and be used to produce efficient vaccines. The importance of a successful vaccination programme for the developing countries is thus more than obvious. Vaccines prepared by recombinant DNA technology, although not yet used in practice, would be expected to have a number of advantages as compared to vaccines produced by conventional methods:

(a) The vaccine will not contain the entire infectious agent, thus eliminating the risks of spreading infections during the manufacturing process or by handling or large volumes of the infectious agent. This problem is of considerable importance, for instance, in connection with production of vaccines against foot and mouth disease virus.

(b) No inactivating processes are required and loss of activity due to deleterius effect of the inactivating processes is avoided.

(c) No test of innocuity of the vaccine is required at least in terms of residual infectivity.

(d) Technically demanding large-scale cultivation of mammalian cells is not required.

(e) Considerably larger quantities of any vaccine could be produced by recombinant DNA technologies. Hence, shortcomings in potency may be overcome by scale of production.

(f) Handling of the vaccine will be less demanding since vaccines can most likely be produced which do not require refrigeration and the same careful handling as conventional vaccines. This will facilitate storage and distribution.

(g) Production costs are likely to be cheaper and the vaccine supply will essentially be unlimited, once a suitable recombinant has been constructed for vaccine production.

To this list of advantages can also be added the fact that recombinant DNA technology will allow production of vaccines against microorganisms which are difficult, dangerous, or impossible to propagate in the laboratory. Important human diseases like hepatitis and malaria belong to this category.

The causes of human and animal infectious disease in the developing countries are bacterial, viral as well as protozoological pathogens. All three categories of pathogens are of considerable importance in most developing countries but it should be noticed that genetic engineering is more easily applicable and potentially advantageous in the manipulation of viruses as compared with bacteria or protozoa.

The principle behind the use of genetic engineering for vaccine production is that one or a limited number of genes from the pathogen in question are inserted into a vector which then is transferred to a suitable host for expression. A prerequisite for the construction of vaccine producing strains is that the gene is known that encodes the antigen or the antigens which induce protecting antibodies. The protecting antigen is known for some viruses and it is then a comparatively simple task to identify the appropriate gene since most viruses contain a limited amount of genetic information. For the more complex pathogens like protozoa this is not the case and considerable amounts of basic research will be needed to identify the appropriate antigen and then to isolate the corresponding gene in order to have it expressed in a recipient cell. Among the problems which require additional work before recombinant DNA technology becomes a general method for vaccine production the following should be mentioned:

(a) The structure and regulation of genes in the more complex pathogens;

(b) Identification of antigen(s) which give rise to protecting antibody;

(c) High-level expression in the recipient of antigens which are active with regard to antibody induction. This will sometimes require modification of the protein and/or cleavage;

(d) Purification of the appropriate antigen(s). This is an important problem since vast quantities of antigen will be needed in many cases;

(e) Procedures for production of stable and potent antigen preparations which give rise to a strong and long-lasting immunity; and

(f) Problems associated with antigen variation.

Ongoing Research

The potential use of recombinant DNA technology for vaccine production was recognized a long time ago and several laboratories are already engaged in the development of vaccines against a few pathogens which are of importance for the developing countries. Among the ongoing projects should be mentioned the production of a vaccine against foot and mouth disease virus (FMDV). The gene which elicits neutralizing antibody against FMDV has been cloned and successfully expressed. A vaccine trial including a small number of cattle has given promising results and the problems associated with the application of recombinant DNA technology to FMDV vaccine production appear to be solved in principle. What remains to achieve are the construction of multivalent vaccines, to optimize expression, to develop simple purification procedures and to prepare potent antigen preparations. There seems, however, to be little doubt that recombinant DNA technology will play an important role for the solution of the FMDV problem.

Hepatitis B provides another important medical problem which has been approached through genetic engineering during recent years. The genome of hepatitis B-virus has been cloned and its structure is known at the nucleotide level although the virus cannot be propagated in tissue culture cells. The surface antigen which is needed for vaccine production provides a more difficult problem to solve than encountered in the case of FMDV since it is a glycoprotein. Expression of the native surface antigen in bacteria is difficult to achieve and proper modification of the antigen will not take place in prokaryotic hosts. The way to circumvent the problem appears to be the use of eucaryotic host/vector systems and promising results have been obtained by using yeast or animal cells as hosts for production of the surface antigen.

Influenza virus is another interesting example since it illustrates the problem of antigen variation. Genes encoding antigens from several influenza virus strains have been cloned in bacteria and the basis for antigenic variation has been elucidated. Multivalent vaccines can probably be produced by genetic engineering procedures although additional work is required to achieve this goal.

In the case of the more complex parasites progress is less spectacular. Very interesting results have been obtained with the trypanosomes and molecular studies of this parasite are advancing with a remarkable pace. Several laboratories in the United States and western Europe are currently working on the molecular biology of malaria parasites. Although quite significant progress has been achieved it appears that the time when specific malaria antigens are cloned and expressed through genetic engineering is still remote. Recent results demonstrate, however, that the project is feasible.

The overall objectives of the work programme on tropical diseases would be fourfold:

(1) to develop a detailed understanding of the mechanisms by which various infectious agents cause diseases. This would be accomplished by studying the life cycles of pathogenic organisms by the new techniques; by cloning and analysing

the genes, by developing procedures to amplify the gene products and by producing monoclonal antibodies against the relevant gene products. These studies then should lead to the development of drugs, vaccines, and various strategies to combat the diseases;

(2) to produce cheaper and better vaccines against pathogens which are of particular importance for the developing countries. The immediate objective will be to select a limited number of pathogens for a detailed study and then in the most rational way to identify the antigens which are useful for vaccine production. In the case of already established means for vaccine production like in the case of FMDV the Centre should immediately solve problems related to pilot scale production, purification and distribution of the vaccine;

(3) to train scientists from the developing countries in the new high-powered technologies so that they can set up programmes to study the diseases which might be of particular importance to their countries; and

(4) to organize workshops and meetings with the objective of increasing communication between the scientists from developing countries and the developed countries and to advance in general our state of knowledge of various diseases and disorders.

Preparation of Work Plan

A careful selection will have to be made with regard to projects at the ICGEB. A larger number of pathogens are responsible for major infectious diseases in the developing countries which also is the case for pathogens causing severe disease in cattle. Many of these pathogens are candidates for vaccine production. A successful

programme must, however be focused on a limited number of pathogens and priorities will have to be made on the basis of feasibility and demand. Tables 1 and 2 list a number of candidate projects. The final dicision as to the selection of projects should be made by the Council of Scientific Advisers when the Centre has been established. Priorities will also have to be made with regard to the choice between projects concerning human versus animal vaccines. In this context it should be mentioned that several institutes in the developed countries have already undertaken efforts to produce vaccines against human pathogens and a certain priority should probably be given to animal vaccines. Except for FMDV and a few similar cases production of animal vaccines will probably turn out to be less profitable and will thus receive a low priority by the technological companies which already have been established in the United States and western Europe. The loss of live-stock severely affects the living conditions for the human population in the developing countries and a successful animal vaccine programme would in many respects have as great an impact as programmes directed against infectious disease in humans. Animal vaccines have in addition the advantage that they require less time-consuming and costly trials before they can be used in practice.

It should also be pointed out that vaccine production by genetic engineering is not always the method of choice to combat infectious disease. Potent vaccines are in some cases already produced by conventional procedures and genetic engineering does not always offer any obvious advantage. It may furthermore be extremely difficult in some cases to construct vaccines against the more complex pathogens, particularly in cases when frequent antigen variation occurs. Therefore chemotherapy should always be considered as an alternative to vaccine production. The vaccine programme as well as the programme for chemotherapy against tropical disease will require substantial amounts of basic research in order to elucidate the molecular biology and the metabolism of the more complex pathogens and these two programmes should hence be intimately co-ordinated. Thus, when considering projects to be undertaken at the ICGEB the following questions must be considered:

(a) Is vaccination a rational approach?

(b) Are there already existing vaccines and in that case are they satisfactory with regard to quality and quantity?

(c) Does genetic engineering provide an obvious advantage as compared to conventional procedures for vaccine production?

A research programme should also be established on schistosomiasis and some other worm diseases with a viewpoint to develop vaccines and diagnostic procedures for these diseases, although the time required to complete these projects may be much longer than five years.

B. ACTIVITIES

The work programme on tropical diseases should have three main components:

(1) research and development;

(2) training; and

(3) workshops and meetings.

Research would involve an in-depth work on a given pathogenic organism at the molecular level, particularly focusing on the mechanism of pathogenesis and of immune response. Studies on the genetic organization of a pathogen, on its surface proteins, on its mode of attaching to the cell surfaces, or on its metabolism would help to develop strategies for prevention (development of vaccines and other preventive drugs), diagnosis (identifying the persons at risk) and treatment (development of drugs and pharmaceuticals). These studies will require laboratories for gene cloning, for cell culture, for DNA and protein sequencing, for DNA and protein synthesis and for making monoclonal antibodies. Thus, a successful programme will require the whole array of modern techniques. One of the main strengths of the ICGEB will be that it will be a comprehensive centre backed up by expertize in molecular genetics and molecular biology so that various important problems can be studied in a concerted manner.

The main question is whether research should be initiated first. It seems that it would not be fruitful for the ICGEB to initiate programmes for cloning of genes for the well-known antibodies and for some well-known hormones (such as insulin). Research in such areas are well advanced. It is recommended that ICGEB consider initiating the following projects and activities:

(a) Genetics of parasites;
(b) Development of vaccines against complex pathogens;
(c) Genetics of some pathogenic bacteria and toxic production;
(d) Vaccines against viruses, such as FMDV, hepatitis, etc.;
(e) Gene bank of pathogenic organisms;
(f) Molecular biology of mammalian cells;
(g) Workshops;
(h) Advisory and additional services;
(i) Alternative procedure;
(j) Diagnostic of microorganisms; and
(k) Clinical trials.

(a) Genetics of Parasites

Parasitology is now at the cutting edge of molecular biology. This is a natural outcome of the progress in the science of molecular biology. The first developments came in bacteria, then yeast became a favourite organism to study and now one can tackle somewhat more complicated systems such as protozoa and thus research work in this area is expanding rapidly and promises to be of great value. Strong research programmes should be set up on complex parasites (worms) and simpler parasites (protozoa).

One important complex parasite is schistosoma mansoni. Some consistent work on schistosomiasis can lead to the development of a vaccine and perhaps a drug to be used in combination with a vaccine. Schistosome infections are initiated by water borne infective larvae released from the snail intermediate host. The larvae penetrate the skin of the mammalian host and undergo a rapid transformation into physiologically adapted parasites called schistosomula. The schistosomula then enter a four week long period of migration and maturation which brings them first via the venous system to the

lungs, where they can be recovered in large numbers and later on to the hepatic portal vessels where the larvae complete their development into adult worms. Once established in the blood stream, schistosomes produce long-lasting chronic infections which in man may extend to decades in duration. During this period the parasites apparently evoke an immune response. A wide variety of different humoral and cell mediated reactions against schistosomal antigens have been demonstrated in chronically infected human and experimental hosts. The laboratory animals bearing adult schistosome worms acquire a partial or total resistance against subsequent infections. Thus, the protective response is induced by a parasite that itself is not damaged by the response. This phenomenon of immunity evasion in schistosomiasis raises two interesting questions.

1) What is the target and effector mechanism of the protective immune response?
2) What is the nature of the adaptation enabling the established parasite to evade this response? The answers to these questions will be important in devising immunological approaches to the control of schistosomiasis and will provide general insights into the problems of parasitic diseases.

Of the protozoan parasites, the malarial parasite is the obvious choice for study at the ICGEB. Work in several laboratories (for example, Wellcome Laboratories, England; New York University, U.S.A.), is progressing systematically and is geared towards finding a vaccine against malaria. Despite the fact that the malarial parasite is an extraordinarily important pathogen for humans, amazingly little is known about the molecular biology of this parasite. The reason for the lack of such studies is that scientists did not have the tools or ways to study the intricacies of organisms more complex than bacteria. However, with the advances in molecular biology, new tools have become available and consequently the field of parasitology is undergoing a revolution.

Of the four malarial parasites, plasmodium falciparum, P. vivax, P. ovale, and P. malariae, only P. falciparum the cause of acute malaria can be cultivated in vitro easily. Its successful cultivation was reported in 1976 and the work on this parasite has been facilitated by this advance. However, work is also continuing on other plasmodia in some laboratories. One of the clearcut projects would be to identify the important antigens in these malarial parasites, isolate the messenger RNA for these antigens, make DNA copies of the RNA and then clone the DNA in E. coli in such a manner that the antigen can be made in bacterial cells. This might lead to a useful vaccine, and to monoclonal antibodies, which can be used immediately to protect people at high risk.

The pathogenic organisms like all other organisms undergo mutations by various mechanisms and become resistant to drugs. Similarily the organisms can also become resistant to antibodies by changing their antigenic structures. Therefore genetic studies at the molecular level have become very important in understanding the nature of the antigenic variation. Thus, genetic studies of plasmodia should be an integral part of the research programme. Some genetic crosses in P. falciparum have been done by injection of parasite of opposite mating types into animals. Perhaps good mating procedures can be developed in vitro and this combined with isolation of mutants would be a good start in understanding the genetics of this organism.

(b) Vaccines against complex pathogens such as the protozoa (malaria, trypanosomes, etc.)

A problem with pathogens like plasmodium falciparum is that they are carried by vectors (in the case of malaria by mosquitos). The pathogen will then appear in different shapes during its life cycle in the human body and in the insect. The different stages of the parasite have different antigenic properties which hamper the development of vaccines by genetic engineering. The genomes of the protozoa are moreover very complex which makes it difficult to identify the gene or the genes which are needed for proper antigen expression. A strategy for development of vaccines against malaria would include the following components:

1. Facilities for growth of the pathogen in sufficient quantities for DNA, mRNA and antigen production;
2. The use of animal models in order to identify the protecting antigen(s);
3. The production of monoclonal antibodies for identification of protecting antibodies;
4. Structural studies of parasite antigens.
5. mRNA fractionation and in vitro translation. Construction of gene and cDNA libraries;
6. Identification of the proper gene(s). This will be a difficult task and several different strategies could be envisaged like selection of mRNA on cloned DNA followed by immunoprecipitation with monoclonal antibodies. Alternatively gene transfer could be used followed by identification of specific surface antigens. Synthetic oligonucleotides could also be used for the identification provided that part of the protein sequence is known;
7. Once the gene(s) are identified a study must be undertaken in order to achieve expression of antigen(s) that are suitable for vaccine production.

(c) Diseases Caused by Bacteria and their Toxins

Two important bacterial diseases are leprosy and tuberculosis. Infantile diarrhoea is also extremely important in developing countries where this disease is an important cause of weakening and death of infants. Several other bacterial diseases are endemic throughout the developing world. One important project would be to develop a diagnostic procedure and protective antibodies against leprosy. The other would be the development of protective antibodies against toxins causing infantile diarrhoea (both vaccines and antibodies would be then available against the two diseases).

For leprosy the research work would include identifying the important antigens and cloning their genes in E. coli, so that the relevant antigens can be made in large amounts. It should be noted that so far mycobacterium leprae, the causative agent of leprosy can only be grown in armadillos found in the southern region of the United States. A combination of protective antibodies and drugs would be an effective way of controlling leprosy. The availability of relevant antibodies will make it possible to devise diagnostic procedures and a more detailed knowledge of the organism may allow us to develop new procedures for growing the organism which in time will allow us to develop new drugs.

The studies on cholera toxin and a related enterotoxin of E. coli responsible for acute diarrhoea in humans and animals have reached a stage where a successful attempt for making a good vaccine can be made. In terms of cholera, the problem has been that whereas natural infection with Vibrio cholerae gives long-term immunity, the existing whole cell vaccines give only limited and short-lived protection. Cloning of toxin genes is an easy technical task. Once cloned, these genes can be manipulated (mutated in vitro for example) to produce toxins that are immunogenic, but not active. Studies involving in vitro site-specific mutagenesis of such toxin genes would be important in many different areas and should be one of the activities of ICGEB.

(d) Production of Vaccines Against Viruses Like FMDV, Hepatitis, Influenca, etc.

A successful solution to the problem of FMDV vaccine production has already been offered. The principle behind the approach is rather simple, since the gene encoding the protecting antigens has been identified. cDNA copies of the viral RNA are inserted into a bacterial vector and expression is achieved by using signals already existing in the vector. The result will in most cases be a

fused protein consisting of bacterial and viral sequences. This particular property does not seem to be a problem but could rather be an advantage for the purification of the antigen. Although bacterial strains have already been constructed which produce FMDV antigen that is suitable for vaccine production, optimization will be needed since massive quantities of the vaccine will be required in order to carry out a successful eradication programme. Thus, alternative host vector systems which are easier to handle, cheaper to grow and which export the antigen into the growth medium will have to be considered. Finally, the immunogenicity of the antigen must be evaluated carefully and preparations will have to be designed which provide long-lasting protection. The latter requirement may involve sophisticated manipulation of the gene encoding the antigen so that an altered protein is produced which is a more potent immunogen than the natural antigen.

The basic strategy for construction of vaccine producing microorganisms is the same as described previously. In cases where the protection antigen is known the appropriate DNA fragment carrying the gene or a cDNA copy of a viral RNA is inserted into a suitable vector in order to obtain expression. Successful expression is monitored by immunoprecipitation. Special care will have to be taken if the antigen is modified by glycosylation of cleavage. In that case the role of the modification on the induction of protecting antibodies will have to be properly evaluated. If the modification is of importance a more complicated strategy must be adopted, involving eucaryotic host/vector systems.

The problem of antigen variation must also be considered. Field studies will in some cases have to be carried out in order to evaluate the role of antigen variation and different strategies will have to be adopted in individual cases.

(e) Gene Banks of Pathogenic Organisms

One of the useful functions that ICGEB can serve is to systematically clone and store complete genomes of various infectious pathogens and their vectors. Such a task will not be undertaken by any other institution or scientists who are merely interested in specific projects. Availability of such a bank at ICGEB will make the Centre a repository of very important biological material. This material will be used by scientists at the Centre and outside.

(f) Molecular Biology of Mammalian Cells

Since sophisticated cell culture techniques will be required in many other projects at the Centre it would be useful to have a small group working on some fundamental aspects of the genetics of mammalian (for example, human) cells. One potentially good project is to study the effects of traditional herbal medicines on the growths and gene expression of mammalian cells.

(g) Workshops

Specialized workshops and general meetings should be an integral part of the programme of ICGEB. These would help promote and speed up vital research and would allow the scientists from the developing countries to communicate with other workers in the field on a continuing basis.

(h) Advisory and Additional Services

An important function of the ICGEB will be to provide advisory services. This activity could be provided in several different ways:

1) Advice as to the choice of projects in laboratories in the developing countries;

2) Advice concerning suitable strategy for problem solution;

3) Technical advice, particularly concerning choice of methods. The Centre should provide laboratory manuals, newsletters, etc.;

4) Advice concerning research tools required to carry out projects.

Genetic engineering is a very demanding technology with regard to consumable supplies. Several expensive reagents like restriction enzymes, autoradiographic film, nitrocellulose filters, short-lived isotopes, etc. are of utmost importance for workers in this field. An important service activity at the Centre will be to produce reagents like restriction endonucleases and to make them available to laboratories in the developing countries which cannot obtain them from commercial sources. Vectors, bacterial strains, oligonucleotides, monoclonal antibodies, etc., should also be provided as a service by the ICGEB. The Centre should also help in distributing rare, expensive, and short-lived isotopes as well as specialized chemicals. A workshop at the Centre could moreover supply custom-made equipment needed for some research activities.

(i) Alternative Procedures

Recently an alternative procedure for the production of specific antibodies has been reported. If the amino acid sequences are known for an antigen it is possible to synthesize a short peptide which is identical with a region in the antigen. Such peptides can be injected into animals for antibody production. Methods are currently available for chemical synthesis of short polypeptides. This procedure has been successfully applied to the production of specific antibodies against some rare antigens. Its potential use in vaccine production has not yet been fully evaluated although results obtained with FMDV appear very encouraging.

(j) Diagnostics of Microorganisms

Genetic engineering can also be used for identification and typing of microorganisms. This is expected to be an essential project at the ICGEB.

(k) Clinical Trials

The clinical evaluation of vaccines should not be undertaken at the Centre. World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) should obviously be involved in this part of the programme.

Training

The Centre should receive and train scientists at the post-doctoral level as well as technical staff. The techniques which are involved in this programme are usually demanding and require a profound background knowledge in biochemistry, genetics, and microbiology. It is consequently important that the candidates are carefully selected for training at the Centre. Another point to take into account is the fact that many activities in this particular programme require a critical mass of scientists in order to be productive.

Therefore it is important that teams of scientists and/or technical staff are trained together rather than single scientists from diverse countries. The Centre should also provide training courses and laboratory manuals which would facilitate the work for beginners. Once activities have been established within the developing countries the ICGEB should provide intensive training courses covering specialized topics.

C. WORK PLAN

The time-table for results produced at the ICGEB will vary for different components of the programme since some tasks that are undertaken will be considerably more difficult than others. Also the target dates when useful products can be anticipated will for some parts be rather unreliable. It is, however, of importance that activities within the framework of the programme will start without delay and results from several activities should be apparent already during the first year. A list of anticipated outputs is given below:

(a) The training programme should start immediately and an annual output in the order of five trained scientists at the post-doctoral level would be expected from the programme. Since, however, the post-doctoral fellows should spend a minimum of two years to receive sufficient training in the major areas of this rather complicated field the first results will be apparent two years after the outset of the programme. Another important output of the programme is the training of technical staff (technicians and engineers) and an annual output of two or three trained technical staff should be expected.

(b) The preparation of laboratory manuals should start immediately and consulting services for already established activities should be provided during the first year.

(c) Training courses and workshops should start during the first year of activity at the Centre. One to two annual courses should be provided by this section of the Centre. Integration between different programmes at the ICGEB is, however, necessary for a successful outcome of the training programme. The workshop programme and expert meetings must also be initiated during the first year. This activity will help to recruit top-level scientists to the Centre. It is furthermore of utmost importance that connections are established with institutes in the developed countries to ascertain high quality

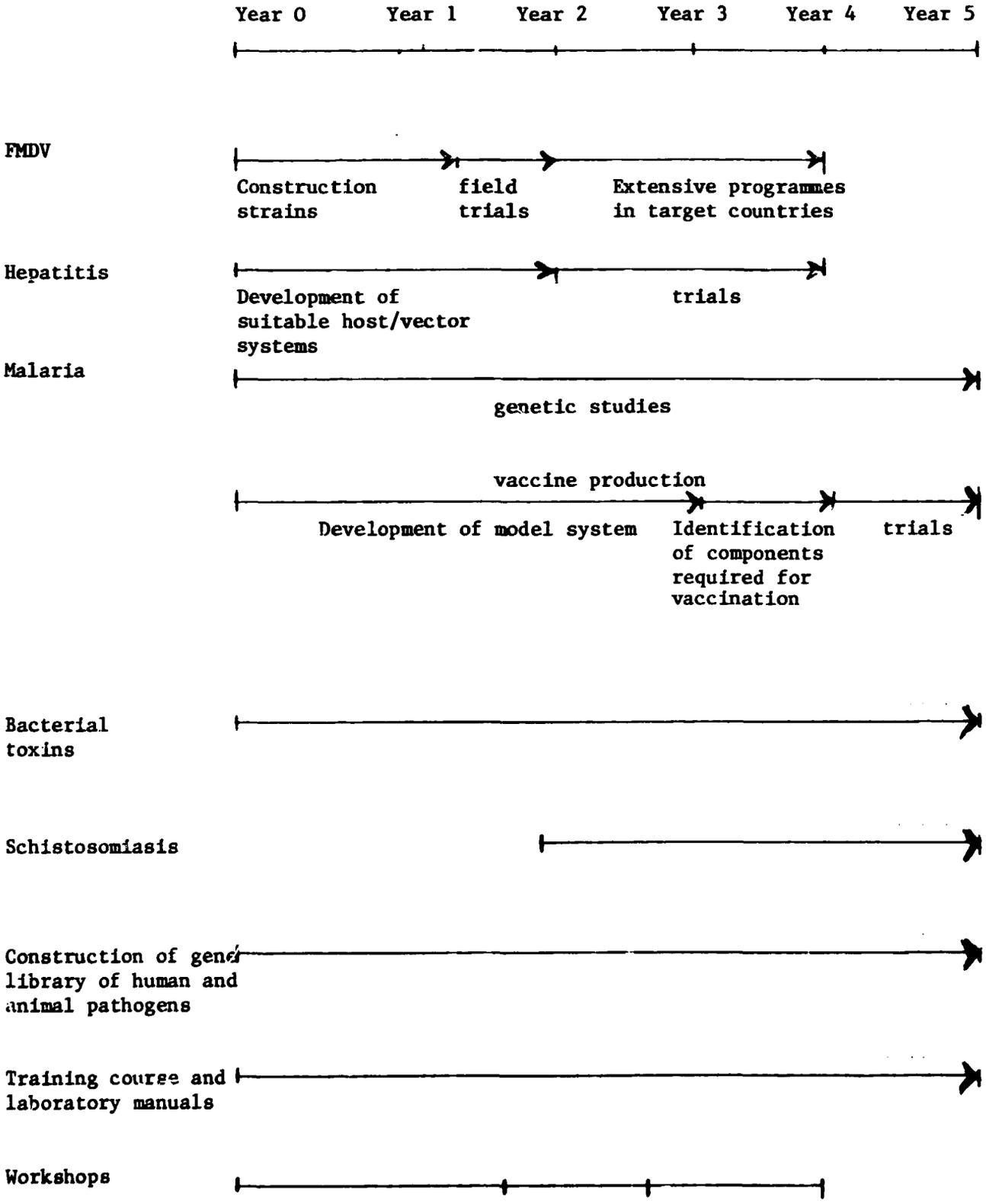
research from the beginning of the programme.

Expert meetings are required to make priorities among different topics to be undertaken at the ICGEB.

(d) Service activities should be started without delay to strengthen already ongoing activities and a separate division should be created to provide research tools such as restriction enzymes, cloning vectors, etc. for laboratories outside the Centre. The Centre should help in providing and distributing commercially available products like short-lived isotopes since many developing countries have difficulties in obtaining these in sufficient quantities today. The Centre could also assist in negotiating favourable conditions for the purchase of expensive reagents.

(e) The most important goal for this programme will, of course, be to construct manipulated microorganisms which produce antigens, suitable for vaccination. The outputs will vary for different projects and it is difficult to make reliable time-tables since the projects which will be given the highest priority at the Centre must be decided by the Council of Scientific Advisers after the ICGEB has been established. The activity within this programme will be expected to focus a few major projects. It is furthermore difficult at the moment to assess the feasibility of the more complicated projects within this programme. It i however, expected that reliable predictions soon can be made concerning several projects listed in tables 1 and 2 since information is accumulating rapidly in this field.

A time-table is given:



D. COLLABORATION WITH OTHER INSTITUTIONS AND INTERACTION WITH RESEARCH AND CLINICAL LABORATORIES

Close association with some laboratories working on some basic molecular biology problems in the United States of America and Europe would help to sustain a high intellectual and technical level. Some obvious choices for interaction are laboratories of members of the advisory group (Ulf Pettersson, University of Uppsala; Ray Wu, Cornell University; A.I. Bukhari, Cold Spring Harbor Laboratory; A. Chakrabarty, University of Illinois; S. Narang, National Research Council, Canada).

The ICGEB must set up close collaboration with the important groups in the developing countries. Some of these groups would be able to provide important technical information and biological materials for work at the ICGEB. Some of the possibilities are as follows:

Leprosy - All India Institute of Medical Sciences, New Delhi;
Malaria - Postgraduate Medical Research Institute, Lahore, Pakistan;
Trypanosomes - International Laboratory for Research on Animal Diseases, Nariobi, Kenya;
Hepatitits - A research group in The People's Republic of China;
Foot and Mouth Disease - A research group in Argentina.

In some cases, the collaboration can be instrumental in greatly speeding up the work. For example, several monoclonal antibodies against Mycobacterium leprae have been prepared in the All India Institute of Medical Sciences. These monoclonal antibodies can be used for identifying and cloning the genes for specific antigens. Given the work already done, a great deal of progress on the problem can be made relatively quickly. Similarly, work on trypanosomes is being done at the International Research Laboratory for Animal Diseases (ILRAD) in Nairobi, Kenya. The research groups at ILRAD may be interested in using facilities at the ICGEB.

Special collaboration will be promoted with ongoing programmes of international institutions, such as World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), United Nations Educational, Scientific and Cultural Organization (UNESCO) and others.

E. PREREQUISITES

As pointed out in a previous section, scientists who are admitted to the Centre for training will have to be carefully selected since genetic engineering work demands profound knowledge of several subjects such as biochemistry, genetics, microbiology, molecular biology, etc. It is an absolute prerequisite for the success of the Centre that very high research standards are maintained at the Centre, not only for the physical output from the Centre, but also if the Centre should succeed in recruiting top-level scientists from abroad. Trainees who are admitted to the centre must consequently be very carefully chosen and the final decision should be made by the Council of Scientific Advisers of the ICGEB. Applicants should not only be accepted on the basis of their academic merits, but also ability to perform skilled laboratory work must also be taken into account.

The future possibilities to establish recombinant DNA laboratories within the developing countries will depend on concentrated efforts. The future need of a particular country must hence be considered in relation to activities going on at the Centre. Also a critical mass of skilled people is needed to successfully establish activities in a new institute that is why it is important to select a group of scientists for training as a team rather than to train single scientists from scattered countries in the world. Selection of candidates for training at the Centre should thus be based on:

1. Academic merits;
2. Documented ability to perform skilled practical work;
3. Experience in relation to activities at the ICGEB;
4. Future possibilities of trainees to establish activities in their native countries;

With regard to selection of technicians and engineers to be trained at the Centre the decisive parameter will be the future need for assistants within a team of scientists in a particular developing country.

F. FINANCIAL REQUIREMENTS

The proposed activity will be very demanding both with regard to personnel, equipment and consumable supplies. The following staff would be required for a successful completion of the programme:

1. Two senior scientists should serve as the co-ordinators of the programme. These persons should have a background in molecular biology or microbiology or preferably in both;
2. Ten assistant scientists;
3. Eight technicians; and
4. Six post-doctoral fellows, most of whom should come from the developing countries. These should have practical experience from recombinant DNA technique or closely related areas.

Among the scientists the following categories should be included: Nucleotide chemist, immunologist, microbiologists, molecular biologists and biochemists. Infectious disease experts, as well as scientists with a veterinary training are needed as consultants.

During the first five years of operation, a total of 20 trainees are expected to benefit from the proposed activities. The following table gives an estimation of the staff and operational costs for five years of operation.

Five-Year Budget

Staff

(First year 40 per cent and second year 60 per cent of full operation)

		(US$ THOUSAND)
Senior scientist	8 man year	600
Junior scientist	40 man year	1,800
Post-doctoral fellows	24 man year	576
Technicians	32 man year	544
Subtotal		3,520
Management of the Centre		233.5
Supporting Personnel		703.5
TOTAL STAFF		4,457.0

Operational Activities

Visiting scientists	64 man months	320
Expert group meetings	4	100
Advisory services	30 man months	300
Training	40 man years	900
Information material		30
Purchase of chemicals, etc.		1,040
Associateship		150
Miscellaneous (travel, telephone, post, etc.)		138
Subtotal		2,978
TOTAL WORK PROGRAMME		7,435

ANNEX I

Table 1

Major Human Infectious Diseases In The Developing Countries For Which Vaccines Need To Be Developed

Malaria
Schistosomiasis a)
Leishmaniasis a)
Filiariasis a)
Trypanosomiasis (sleeping sickness) a)
Leprosy
Tuberculosis
Cholera b)
Toxin-producing enteric bacteria
Trachoma
Hepatitis B b), c)
Rabies
Hepatitis A
Poliovirus b)
Rotaviruses
Arboviruses (Yellow fever, Encephalitis, Rift valley fever, etc.) b)
Influenza b)

a) It is not yet known in all cases whether vaccination with genetically engineered vaccines is feasible.

b) Vaccines, produced by conventional methods, are available but it is expected that genetic engineering could be applied to produce improved and/or cheaper vaccines.

c) Promising results available for application of genetic engineering.

Table 2

Major Animal Diseases in Developing Countries

An Assessment Of The Potential Of Genetic Engineering For The Improvement Of Vaccines

Disease	Category of Importance	Priority for genetic engineering in vaccine preparation
A. Viral Diseases		
Foot-and-Mouth disease	1	High
Rinderpest	1	Low
Rabies	1	High - bat-transmitted rabies in cattle
Vesicular stomatitis	2	Medium
Bluetongue	2	High
Hog cholera	2	Low
African swine fever	2	Unknown - high as a research tool
African horse sickness	2	High
Venezuelan equine encephalomyelitis	2	Low
Malignant catarrhal fever	3	Low
Pulmonary adenomatosis	3	High
Aujesky's disease	3	Medium
B. Viral Diseases of Poultry		
Newcastle disease	1	Medium
Marek's disease	2	Low
C. Bacterial Diseases		
Tuberculosis	1	High
Brucellosis	1	Low
Clostridial infections	1	Low
Anthrax	2	Low
Pleuropneumonia	3	Low
Leptospirosis		
D. Protozoal Diseases		
Trypanosomiasis		These diseases, especially the first two, are of great economic importance in Africa. The progress of research has not yet arrived at the point at which vaccination is feasible. This will come but when it does, many consequential problems will have to be evaluated before deciding upon the correct strategy. (Source: 'The Potential of Genetic Manipulation for the Improvement of Vaccines Against Animal Diseases in Developing Countries', UNIDO/IS.273)
Theileriosis		
Anaplasmosis		
Babesiosis		
Coccidiosis		

ANNEX II

EQUIPMENT REQUIREMENTS

When fully developed the programme should include all modern techniques which are of importance for id ntification and expression of genes in different host vector systems. Such techniques will include:

(a) Facilities and procedures for growth and purification of the pertinent microbes. This is not a trivial problem for many microorganisms which are of particular importance for the developing countries;

(b) Basic techniques for molecular cloning, i.e. techniques for construction of DNA libraries, cDNA cloning, etc. Cloning methods using both E. coli, B. subtilis and yeast should be estabished as routine procedures;

(c) Techniques and reagents for identification of the relevant antigens, i.e. polyclonal and monoclonal antibodies, animal models, etc.;

(d) Techniques for identification of the relevant gene products, i.e. in vitro translation in combination with immunopreceptation;

(e) DNA sequence analysis;

(f) Methods for chemical synthesis of oligonucleotides;

(g) Electronmicroscopy for studies of nucleic acids;

(h) Vector technology. New and improved encaryotic host vector systems are likely to be required for production of efficient vaccines. Less demanding and inexpensive host/vector systems which are suitable for use under primitive conditions also need to be developed;

(i) Procedures for gene transfer, i.e. microinjection and transfection. Immunofluorescent methods;

(j) Tissue culture techniques;

(k) Methods for large-scale cultivation of microbes and purification of antigens. This problem should be approached in close contact with other programmes at the ICGEB; and

(l) Techniques and facilities for purification and preparation of stable and potent vaccines.

The following equipment will therefore be required:

Heavy equipment required by the unit would include:

4 ultracentrifuges;
4 medium-speed centrifuges;
5 Eppendorf centrifuges;
4 other table top centrifuges;
1 icemachine;
5 water baths;
2 lyophilizers;
2 scintillation counters;
1 gamma-counter;
electrophoresis equipment including power supplies for DNA sequencing;
3 balances;
2 freezers (-70^{o});
fluorescensce and light microscopes;
incubators;
spectrophotometer;
HPLC;
pH-meter;
vacuum oven; and
facilities for tissue culture and cultivation of bacteria.

Table 1

Facilities And Equipment To Be Shared With Other Groups At The ICGEB

- Dish-washing and kitchen facilities;
- Facilities for preparation of media (animal cells, protozoa and bacteria);
- Tissue culture facilities;
- Containment (P3) laboratory;
- Electronmicroscopy unit;
- FACS-unit;
- Protein sequencing laboratory (amino acid analysers, sequenators, etc.);
- Oligenucleotide synthesis unit (HPLC, NMR equipment, etc.);
- Facilities for peptide synthetis;
- Unit for production of monoclonal antibodies;
- Animal facilities;
- Dark room and photographic equipment;
- Computer with accessory equipment; and
- Word processers.