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Genetic Engineering and Biotechnology in Nepal

Report to UNIDO

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PREAMBLE

I would like to thank most sincerely the members of the staff of the Royal Nepal Academy of Science and Technology for their many kindnesses during my short visit to Kathmandu in December 1989. I want especially to say how much I valued the help and advice of Dr. Keshari Manandhar, Associate Member of RONAST, who has done so much already to discuss genetic engineering and biotechnology in Nepal. She organised my visit and facilitated my work in every way possible.

INTRODUCTION

Objective

The objective of the mission was to investigate the current state of knowledge and practice in genetic engineering and biotechnology (GEB) in Nepal and to advise whether and how GEB could be applied to support economic and social development in Nepal.

Duration of the mission

The mission took place during 7 days in Nepal in December 1989 between arrival in Kathmandu on Wednesday 13th and departure late on Tuesday 19 December. The project was discussed briefly with officials of UNDP (Appendix I).

Host organisation

The Royal Academy of Science and Technology (RONAST) organised the schedule of the mission, providing office accommodation and transport. Dr. Mrs. Keshari L. Manandhar was responsible for arranging the meetings and provided background documentation and advice.

Institutions visited

The mission was concentrated in Kathmandu and the neighbouring region; the major technical and scientific institutions of Nepal are located there. Visits took place to:

Royal Nepal Academy of Science and Technology
Royal Drug Research Laboratory.
Central Animal Disease Investigation and Research Laboratory.
National Agricultural Research and Service Centre.
National Herbarium and Botanical Gardens.

Teaching Hospital.
Central Food Laboratory.

Background

Nepal is certainly a most beautiful country, with a great range of climate and terrain, matched only in the variety of the people and culture. It covers just over 55,500 square miles, about twice the size of Ireland, shaped roughly as a rectangle 500 miles long by 100 miles wide. The land, rising from close to sea level on the Ganges plain to the Himalayas, is divided by many mountain ranges and plateaus, and traversed by many rivers. Several of the great rivers rise in Tibet and all eventually flow into the Ganges. Nepal is essentially a mountainous country, with 65% of the territory lying above 3000 feet. 10% of the land lies above the snowline, including of course Sagarmatha, Kanchenjunga, Annapurna in the great expanse the high Himalayas. Communications are extraordinarily difficult.

The population of Nepal is more than 18 million, nearly 5 times that of Ireland. In 1977 the population was 13.5 million. Currently it is growing at a rate of 2.7%. Almost all the people (94%) are engaged in agriculture. Agricultural produce accounts for 60% of the Gross Domestic Product and 75% of the exports. Agriculture is of course practiced throughout the country, but only 15% is under cultivation; much of the remainder is either mountain or jungle. The variety of climate and terrain has ensured that almost every kind of crop from tropical to temperate is grown somewhere in Nepal. Much farming is at or close to subsistence level. There are however some areas of very great fertility, including especially the tropical region (the Terai) along the Indian border where the landscape falls down to the Ganges plain, and the plateaus and valleys in the centre of the country, especially the Kathmandu Valley. The valleys are quite small (the Kathmandu Valley is only 600 square miles) but there are many of them. Ingenious terraces have turned the hillsides into fertile fields. The main crop is rice, accounting for about half of all the grain, and this is followed by corn (25%), wheat (10-15%), barley and millet. Potatoes and many kinds of fruit, vegetables and pulses are also grown. Whil Nepal is self-sufficient in food and a net exporter of food, it is vitally important to increase production both for export, to provide for consumption by the steadily rising population and to improve the average level of nutrition.

There are many peoples in Nepal of different ethnic origins who speek a number of languages and many more dialects. Nepali is the official language. It is a sanskrit language and is spoken by more than 50% of the people. Most of the rest of the population speak other languages related to sanskrit and thus to the dialects of north India. A

small number (about 5%) speak the Newari language which is important partly because it is spoken by many people in the Kathmandu Valley. Newari is related to Tibetan. There are many other Tibetan or Tibeto-Burman languages and dialects, some spoken by large groups such as the Gurungs of the Western Hill region, and the Chepangs. Others are spoken only by small groups localised in isolated valleys.

Nepal, dedicated as a Zone of Peace, is one of the least developed nations. It is rapidly becoming affected by outside cultures. Lying between the great nations of China to the north and India to the south, it is subject to strong influences from both. It has close ties with other countries of south Asia, with many European and North American countries and it has growing links with Japan. The Government is committed to social and economic development. It must solve very great problems if there is to be a smooth transition from the shimmering quilt of traditional cultures to "modern" society, which to the unquestioning mind is believed to be more desirable in every respect. The remarkable and often exquisite features of the native cultures of Nepal are now being severely challenged by the strong and usually crude economic and cultural forces which are coming from outside. These external pressures are following inevitably in the wake of trade, tourism, communications, education and industrialisation. Nepal is striving to ensure that the best characteristics of its own cultures are preserved while it assimilates the benefits of western society. In addition it faces the daunting strategic task of becoming more self-sufficient in energy and industrial materials and products. Today Nepal is wholly dependent on the importation of most raw materials, oil and industrial goods, by road from India.

THE ROLE OF GENETIC ENGINEERING AND BIOTECHNOLOGY IN DEVELOPMENT

Since 1970 there has been remarkable interest worldwide in the applications of genetic engineering and biotechnology. The question is what impact GEB can make in Nepal, and how this should be organised.

The two terms of "genetic engineering" and "biotechnology" need to be defined. They are often linked together and are sometimes used interchangeably. They are of course related but not in the logical way that one might expect from usage. Biotechnology is the industrialised use of biological systems for economic or social benefit. This definition usually excludes traditional agriculture. It includes many ancient manufacturing processes which have been modernised through the use of technology. The ancient biotechnologies used to be practiced on a family or village scale and they include the manufacture of many processed foods and drinks, usually through microbial fermentation. Examples are the making of bread, beers, wines, cheeses, yoghurts, soy sauces, pickles

etc. The retting of linen, the fermentation of certain kinds of tea, the making of dyes such as indigo, the tanning of hides and the preparation of many indigenous foods, which all involve microbial processes, have increasingly been organised on an industrial scale in the modern world.

Biotechnology received a major boost with the realisation in the 19th century by Pasteur and others of the nature of microorganisms and their roles in making foods and drinks. Once pure cultures of valuable microorganisms had been obtained, and once it was realised that they could be grown, studied and used as such, the way was open to exploit them more effectively in the food and drink industries. Thus the first large scale applications of modern biotechnology were in the beer and spirits industries. The large breweries and distilleries, and the new large scale chemical plants of the late 19th century, were to pave the way for the first use of microbes in the production of organic chemicals such as acetone and butanol, though these processes (with exception of the production of ethanol and acetic acid) have never become widely used. The next major boost for biotechnology was the discovery of antibiotics and the development of the large scale production method for penicillin during World War II. Fermentation and downstream processing methods became much more sophisticated and eventually became the subject of a recognised discipline, process engineering.

The post-war growth of the biotechnology industry up to 1970 was essentially related to the growth of the antibiotics industry. It was a matter of diversifying, through the discovery of more and more antibiotics, automating and scaling up. There was also, to a lesser extent, some development of new methods of medical diagnosis. There was also a steady growth in the production of industrial enzymes related to the growth in the production of processed foods and drinks. No revolutionary scientific or technological ideas were involved.

In 1970 two discoveries were made which brought biotechnology into great prominence. First, a new technology called genetic engineering emerged from the science of molecular genetics, largely through the work of researchers in the United States and Western Europe. Second, a way was found to make a more purified form of antibodies, called monoclonal antibodies. The monoclonal antibodies were produced from tumours called hybridomas, which were first constructed by two immunologists at Cambridge.

These two different scientific developments immediately suggested methods for manufacturing valuable products, some of which had never been made before in sufficient amounts to be useful. Genetic engineering and monoclonal antibody technology offered significant prospects for revolutionary change in biotechnology.

Genetic engineering is a set of methods for isolating and analysing genes, and for transferring them from their natural host to other organisms where they can be incorporated with the genes of the recipient organism and be passed on to future generations of the new host. The consequences of this are immense and can be illustrated by taking one example.

Genes act by causing proteins to be made. Each gene is said to code for a particular protein. If a particular gene is present in an organism, then the protein coded by that gene can be made in that organism. Insulin is a protein. If the gene for human insulin is isolated (and this is difficult because a human has somewhere between 100,000 and 1,000,000 genes) and put into another organism, say a yeast, (in a form called a cDNA gene) then the yeast will (under certain precisely defined circumstances) begin to make human insulin. Such a genetically engineered yeast can be grown on a large scale, using pharmaceutical fermentation and downstream processing plants, and large amounts of human insulin can be made. This and similar results have been achieved by several pharmaceutical companies notably Eli Lilley and Novo-Nordisk and human insulin is now available for the treatment of diabetics. Likewise interferons (coded for by different genes) are being made from genetically engineered microorgansisms for use in cancer chemotherapy and the treatment of viral diseases, growth hormone (another protein coded for by yet another gene) is being made for treating dwarfs (and also burns), clotting factors are being made for treating haemophiliacs, and anticlotting factors are being produced to treat those at risk of heart attacks and strokes. Genetic engineering has opened up the prospects of many new drugs for the pharmaceutical companies.

Genetic engineering is quite revolutionary, and goes much further than this. It can be used to make entirely new forms of vaccines for either human or animal diseases. Genes from viruses and protozoans (such as the parasites for malaria and sleeping sickness) are being transferred to bacteria and yeasts to make these novel and completely safe vaccines. The first genetically engineered human vaccine to come on the market is for Hepatitis B. It has been made by Merck and also by Eli Lilley, and more recently in China. Some projects have been slower and less effective than originally hoped for, but there are very good reasons to believe that many new vaccines will be produced by genetic engineering.

Genetic engineering can be used to make industrial enzymes both in a greater range and more cheaply. These are the enzymes used in industrial processes (brewing, distilling, cheese making, bread making, sizing cotton, clarifying fruit juice and wine etc.) and other products

such as detergents or high fructose syrups, for example. Novo-Nordisk has just introduced a novel genetically engineered lipase in both Japan and Europe.

Industrial microorganisms, which are used in manufacturing processes (antibiotic synthesis, organic solvent and acid production, brewing, oil recovery, cleaning etc), can also be genetically engineered to be more effective. There are interesting prospects in genetically engineering Streptomyces species to make novel antibiotics which have been opened up by the work of Hopwood at the John Innes Institute in Norwich. Plans to modify bacteria for use in tertiary oil recovery have not been followed up as rapidly as was once mooted, partly because of the fall in oil prices. Other plans to use genetically engineered yeasts in brewing and distilling have been set aside for the time being due to concerns of public acceptability. On the other hand the Brazilian gasohol project is continuing to develop strongly and this may be a sphere in which genetic engineering is applied usefully in industrial yeasts. Japanese scientists have recently developed a new microbial process for manufacturing acrylamide. The US high fructose syrup industry which produces fructose from corn starch by immobilised enzymes and/or microorganisms, dominates the production of sweeteners there. We may see genetic engineering being used in each of these cases to improve the strains.

Agriculture is a most promising area for the application of genetic engineering. The first genetically engineered vaccine was in fact against scour in pigs. More recently there has been success in producing virus-resistant plants by inserting virus coat protein genes into plant genomes. Other plants have been made resistant to insect pests by the insertion of a Bacillus thuringiensis BT toxin gene. In a different example plants normally susceptible to herbicides (such as Roundup) have been made resistant to it by the transfer of a bacterial gene into the plant genome.

One other area which has been remarkably affected by genetic engineering is the whole matter of diagnostics. One can use DNA probes to diagnose a genetic disease in man, a virus disease in a plant, or a protozoan infection in a farm animal. These DNA probes are made by genetic engineering or by formulae which have been obtained by genetic engineering. The same kind of probes are used now in forensic science to identify criminals by genetic fingerprinting.

The range and applications of genetic engineering is thus very wide indeed. The few examples given above are evidence that it can be used in virtually all kinds of biological systems and therefore it can be incorporated into the wider technology of biotechnology.

Monoclonal antibodies: Monoclonal antibodies do not have the same wide implications as genetic engineering. They were significant however in adding to the sense that biotechnology was going into a new phase, and they quickly gave rise to profitable new enterprises. They are used in diagnostics and there are ambitious schemes to use them to direct drugs to specific tissues for example tumours.

The new logic of biotechnology

Genetic engineering (GE) changed the logic of biotechnology. Before GE new biotechnologies depended on discovering new biological processes which could be operated on an industrial scale. Mutagenesis and process development could occasionally be used to improve the efficiency of a new process, but there were stringent limits to innovation, and most desirable processes or products could not be developed due to problems of scale or cost or both. With very rare exceptions (where mutagenesis produced novel processes) a new biotechnology had to be carried out in the organism where the basic process existed naturally.

GE opened up a revolutionary strategy. It can be summarised as follows. First identify a desirable process or product in nature. Then use GE to transfer the genetic system coding for the process or product to a wellknown industrial or agricultural organism, and modify it so that it can be used economically. In addition GE can be used, for example through protein engineering, to develop entirely novel biological processes and products which do not exist naturally.

The response in developing countries

GE was developed in universities through a series of discoveries about the molecular genetics of the bacterium Escherichia coli. All the essential basic discoveries (DNA ligase, Type II restriction enzyme, DNA polymerase I, reverse transcriptase, plasmids) had been made by 1970. They were first put to use in scientific experiments on fundamental scientific questions in the early 1970s. After a slow start, the field accelerated from 1975 onwards, and the techniques of GE rapidly spread throughout the university world and into industry. By1985 GE was being used to make striking advances in every field of biology. Today molecular genetics is an essential component of biology courses in university undergraduate curricula, and the techniques of GE are taught to those undergraduates who specialise in genetics, biochemistry, microbiology and molecular biology.

The first attempts to commercialise GE took place in the mid 1970s. Genentech, the first GE company, was founded in 1976. Many

other small companies were established in the United States and Europe funded by venture capital. A few prospered and have survived to become small to medium sized pharmaceutical companies. Many others have been incorporated into larger companies. The first GE product was human insulin. This has been followed by about 20 others.

GE is now part of mainstream biotechnology, an integral part of virtually all biotechnology research and development. It comprises a set of distinctive experimental skills which are practiced alongside genetics, microbiology and biochemistry in almost all companies which are carrying out research in biotechnology.

The UNIDO programme in biotechnology

UNIDO initiated a series of discussions about GEB and developing countries in the early 1980s. International missions were sent to many developing countries. It was found that there were many opportunities to apply GEB but that there was an almost complete lack of knowledge of the subject of molecular genetics and no experience of GE technology. An international conference in Belgrade led to the proposal that an International Centre for Genetic Engineering and Biotechnology should be set up to facilitate the introduction of GEB to developing countries. The ICGEB has been established with two laboratories in Trieste and Delhi.

The impact of GEB

Clearly genetic engineering and the new biotechnology is expected to have considerable importance for developing countries. This is certainly true though probably not in the way that most people have suggested.

It is not easy to convince governments to undertake new programmes, especially when there are already many other very important programmes which are not properly funded This is especially the case in the developing countries, where the basic economic and social problems are so demanding.

Various suggestions were made to encourage the introduction of biotechnology programmes in developing countries, some of them emphasising the benefits and some the threats of GEB. For example it was quickly perceived that it might be possible to develop new vaccines against malaria. The first attempts for a vaccine have not been successful. But GE has led to significant advances in the understanding of malaria, and there are interesting prospects for using GE to design new antimalarial drugs. If malaria is eliminated or greatly reduced then all the claims for GEB will have been satisfied. The fact that there are

genetically engineered hepatitis B vaccines, which are believed to be very effective, is a major advance. But the cost of a course is relatively high and they have not yet been introduced on a large scale in the regions of Southeast Asia where hepatitis B (and therefore liver cancer) is endemic. The Institute of Biochemistry of Academica Sinica has a hepatitis vaccine.

It will be important if the HBV vaccine can be effectively distributed on a large scale at low cost in developing countries since this would illustrate the importance of GEB.

There are numerous other examples of projects which have not quite borne out the promise which was advanced for them when the discussions of GEB and the developing world was initiated. However the benefits have not been overestimated, only the timescale has been underestimated.

It is important to put GEB in perspective, and the best way to do this is consider what has been achieved in developed countries. It has had a huge impact on research and teaching in virtually all areas of biology, including medicine and agriculture. There have also been major breakthoughs in the introduction of new pharmaceuticals. But GE has not quite given the expected social and economic benefits in developed countries, at the level which was anticipated by the flood of venture capital investment in the period from 1975 to 1985. But even if the initial enthusiasm in the United States and Europe was exaggerated, noone is any doubt that GE is bringing a remarkable increase in knowledge about many important biological systems and that it has changed the way in which biotechnological projects are conceived and carried out. For example without GE we would have a very poor understanding of AIDS and little or no hope of finding either a cure or a vaccine. After a studied and properly sceptical assessment of GE, the major pharmaceutical companies now invest heavily in GE. This is the best possible single reason for developing countries to continue to take GEB seriously. Noone should doubt that there will be many more benefits, the only question is when.

Fortunately, while some of the benefits have not yet materialised none of the threats have. Consider two examples. At one time it was anticipated that new biotechnological meth ds of tertiary oil recovery and large gasohol projects based on starch or cellulose would reduce the demand and therefore the price of oil, a major product of some developing countries. Neither threat has had any effect so far. The main effect on the price of oil has been the increased efficiency of the developed economies in response to the high price of oil. But the question remains whether biotechnology will ever have any effect on oil

demand. There is no answer to this at present. We should be cautious. The demand for sugar was hugely reduced by the development of the biotechnological process for making high fructose syrup, and this involved the commercialisation of the activity of a single enzyme, glucose isomerase. If new methods can be developed for efficiently hydrolysing waste cellulose, then oil could be affected.

The question of whether developing countries should pay attention to GEB must be answered in the affirmative. Molecular genetics, the science which encompasses GE, is now central to the study of biology, including medicine and agriculture. Molecular genetics now holds a similar place in biology to biochemistry. GE is also central to modern biotechnology. To date it has only led to a about 20 remarkable products, but there are many in process of development.

GENETIC ENGINEERING AND BIOTECHNOLOGY IN NEPAL

Introduction

Nepal faces a very difficult task in dealing with the subject of GEB. GEB is essentially a technological form of biology. It will be useful to consider it as it affects industry, medicine and agriculture, and later as it relates to training, education and research. I am grateful for being given access to a number of papers and reports including the discussion by Dr. Manandhar presented at the International Symposium on Application of Biotechnological Methods and Recent Accomplishments of Economic Value in Asia, Bangkok, November 1989. She drew attention to opportunities in biogas, biofertiliser, plant tissue culture, post harvest loss and mushroom culitvation. Nepal has made a good start in some of these areas and I will refer to some below.

GEB and industry in Nepal

Since Nepal is mainly an agricultural country, with little industry, one should not expect to see immediate prospects for novel kinds of industrial biotechnology incorporating for example the new technology of genetic engineering. None of the areas mentioned by Dr. Manandhar refer to industrial biotechnology. While there are of course many traditional biotechnologies being practiced on a family and village scale, very few of these have been developed to a significant degree. Here one needs to consider the development of such industries as bakeries, distilleries and breweries. They are being set up in Nepal and have considerable prospects of success. They will play a significant role in the longterm growth of a biotechnological industry in Nepal. They are the first steps on the way to modern biotechnology. They lead to the introduction of good microbiological practice, quality control, large scale fermentations

etc. But they will not be the first places in Nepal to be affected by the new biotechnology. The main reason for saying this is that none of these industries has introduced new biotechnology anywhere in the world. The new biotechnology has not led to remarkable innovation in brewing, distilling or baking - it is expensive to apply and the economies are small. Moreover the public, for no good reason it should be said, has been reluctant to accept the idea of beer or bread being made by genetically engineered yeasts. So the bakeries and breweries cannot be seen as likely places for the introduction of GEB in Nepal.

The pharmaceutical industry is the sector which has made most use of GEB in developed countries. Pharmaceutical GEB projects typically last for 5 - 10 years, they are usually manned by 10-100 people, and they cost a minimum of $250,000 to finance. They are usually aimed at international markets valued at more than $100 million per year. It is not surprising that GEB has been applied only by multinational companies which have great experience in research and development. Occasionally a small company will use GEB to make a startling discovery of great commercial value but this will be rare, and will not be developed, or passed as safe by the regulatory authorities or marketed, without the help of a major multinational company. It can be seen that, with a few exceptions, GEB will not be applied by the pharmaceutical industries in developing countries for many years to come.

Very few drugs are made in Nepal. I was informed that some are formulated from imported raw materials. There is very little pharmaceutical research in Nepal. I visited the Royal Drug Research Laboratory. It is not constructed, equipped, financed, staffed or organised in a way which would allow for the conduct of the kind of research which involves GEB. No plans should be made to introduce GEB for pharmaceutical research or development in Nepal.

GEB and medicine in Nepal

The science of molecular genetics is leading to considerable advances in medical knowledge and new products and diagnostic procedures are being introduced through GEB.

But, that having been said, it is important to remember that about 80% of medical treatment is relatively routine, requiring surgical or medical procedures which have been well established for at least 20 years. The main objectives in Nepal are to develop sufficient hospitals and clinics, and to train sufficient doctors, nurses and support staff to bring "ordinary" medicine within reach of all the people. This task will not be affected by GEB, except in a few respects which I will mention here.

Vaccines There are new vaccines and drugs made by GEB, and there will be many more. The vaccines present real possibilities for major improvements in public health. I was informed that Hepatitis B is becoming a problem in certain parts of Nepal. Strenuous collective efforts should be made by the developing countries through the World Health Organisation to ensure that the HBV vaccine is introduced at reasonable cost. There are likely to be other similar cases. Nepal should use every opportunity to seek international support for the introduction of these new vaccines through WHO.

Drugs At present the new GEB drugs are very expensive and are contributing to major problems in financing medical care in developed countries. Though these drugs may be helpful in some cases they are so expensive that public health authorities are finding it very difficult to decide whether they can be justified in terms of the overall needs of the people and the limitations on the health budget. Nepal needs to be cautious about introducing these new drugs until they have been proven in value and they are available at reasonable costs.

Diagnosis Novel methods of diagnosis have been developed by GEB using either monoclonal antobodies (MAB) or DNA probes (gene probes). These are likely to become very widley used in diagnosing viral or microbial pathogens as well as cancers. The MAB technology is an extension of relatively wellknown immunological procedures and should be easily assimilated in any immunology laboratory. The DNA probe technology including the Polymerase Chain Reaction relies on completely different concepts with different procedures and equipment. I believe that one or perhaps two pathologists should obtain training in the use of DNA probes to prepare for their use in Nepal. This is not an urgent matter compared to the other demands on the Health Services and it may well be that young doctors being trained abroad will acquire sufficient knowledge in due course. I visited the Pathology Laboratories of the Teaching Hospital. This institution is well suited to introducing DNA probes to Nepal.

GEB and agriculture

Agriculture is undoubtedly the area in which GEB has the most obvious application in Nepal. GEB is likely to have large effects on agriculture worldwide, and of course agriculture is the basic economic activity of Nepal. Moreover there are several agricultural research stations in Nepal and there is a core of experienced researchers some of whom are already skilled in some of the basic skills required for the application of GE to agriculture. I was most interested to meet several researchers at these stations.

I visited research laboratories with projects related to agriculture at the Royal Nepal Academy of Science and Technology, the Central Animal Disease Investigation and Research Laboratory, the National Agricultural Research and Service Centre, the Central Food Laboratory and the National Herbarium and Botanical Gardens. I was informed about some of the problems which have affected Nepalese agriculture. I saw direct evidence of good quality classical research on some of these, and it was obvious that GE could be used to improve this work. I will give some examples.

The Biofertiliser project of RONAST

Biofertiliser is obtained from the use of inocula of bacteria of the genus Rhizobium. These organisms form symbiotic relationships with the roots of leguminous plants such as soybeans, peas, beans and pulses. They cause atmospheric nitrogen to be fixed in such a way that it can be used by the plant as fertiliser instead of nitrates. Nitrogen fixation is a natural process which costs virtually nothing by comparison with chemical fertiliser.

Given the great difficulties of transporting chemical fertiliser such as nitrates to remote or hilly regions, it is of great economic importance to ensure that Nepali farmers make full use of biofertiliser through the nitrogen fixing organisms Rhizobium. Dr. Kayo Yami and her colleagues have worked on this area for several years, in association with the Department of Agriculture. They have been screening natural isolates of Rhizobium to find good combinations of bacterial and plant strains. Their work has been funded by the International Foundation for Sciences and the Food and Agricultural Organisation. The FAO project will run to 1991. Work at JIRI Agricultural Research Station has shown that soybean seeds which have been coated with Rhizobia give 40-60% better yields than those which have not been coated. Now they need to match more strains and varieties, to mass-produce the inocula, and to coat, market and distribute the seeds.

The immediate use of GEB in this work is in strain identification. Dr. Yami needs to collect, identify and characterise many strains which must be stored reliably and retrieved easily. Large numbers of strains can be easily and reproducibly characterised by either plasmid DNA profiles or by genetic fingerprinting to reveal restriction fragment length polymorphisms. These methods are not expensive and are becoming standard procedures in microbial identification worldwide. They are very similar to those used in medical and veterinary diagnostics. When Dr. Yami gets experience in this field her knowledge will be invaluable to her colleagues in medicine and veterinary medicine.

Reafforestation

Reafforestation is a priority for Nepal. Plant tissue culture has a crucial role to play in this through rapid micropropagation. The Tissue Culture Laboratory of the National Herbarium and Botanical Laboratories at Godawary were most impressive. I learned from Dr. Rajbhandary of his novel method for rooting microshoots of many different species including some major forst trees which he has presented at the 6th SABRAO Conference in Japan in 19[illegible]. Although I am not an expert I was most impressed by his procedures. Any GEB programme in Nepal should include a commitment to test the application of his work in field trials.

The potato tuber moth

The potato tuber moth is a pest which has reached Nepal quite recently. I was informed that it was indigenous to America, but has since been transmitted from there to Europe, India and now Nepal. It destroys potatoes, including seed potatoes, in storage. It has been shown at the NARSC that foliage from various aromatic wild plants protects the potatoes. From what I could learn in a brief visit this seems to be both cheap and effective.

An alternative strategy occurred to me which would involve GEB. Spores of the bacterium Bacillus thuringiensis contain toxins which is lethal for members of the Lepidoptera (moths and butterflies). Genes for these toxins (called BT) have been isolated by cloning and put into plant genomes by GE. The genetically engineered plants have been shown to be resistant to Lepidoptera.

Potatoes are wellknown organisms for tissue culture (and this is important for plant genetic engineering), Nepali scientists are skilled in potato tissue culture, and the genes for BT toxin are available from university research laboratories. Thus there are good reasons to use this project as a test project in cloning in Nepal.

Citrus greening disease (CGD)

Dr. Regmi is an expert on CGD. I was informed that Nepal used to be a net exporter of citrus fruit. Now it imports about 1000 tonnes of citrus per annum from India. One reason for this is Citrus Greening Disease, an international problem which is causing much discussion. It is transmitted by a number of insect vectors including the homopteran Diaphorina citri and the hymenopteran Tetrastichus radiatus. There is a UNDP 5 country programme on CGD for China, the Philippines, Thailand and Indonesia which is coordianted by Dr. Aubert. I understand that it has been very difficult to establish the cause of CGD, and it is extremely

difficult to show whether stocks of citrus are infected. It is vitally important for Nepal to ensure that as many areas as possible are kept disease free. This means that new stocks of citrus must be tested for CGD. French laboratories at the University of Bordeaux II have raised monoclonal antibodies against CGD and these may prove to be helpful.

An alternative strategy for testing is to find DNA probes for CGD and use the Polymerase Chain Reaction. It is certain that this will be tried under the aegis of the FAO, or the International Organisation of Citrus Virologists, but Nepal should be prepared to introduce it as soon as it becomes available.

If CGD is found to be caused by a virus then it may be useful to try to genetically engineer citrus to virus-resistance. This would be a much more complex project.

Mushroom cultivation

The work at the National Agricultural Research and Service Centre on mushroom cultivation was started in 1975. A service has been established which supplies farmers with mushroom spawn. I understand it has given rise to a new cash crop. This is an excellent example of a project which has reached the stage where it could probably be commercialised.

Veterinary diagnosis and vaccination programmes

I visited the Central Animal Disease Investigation and Research Laboratory. I greatly enjoyed the discussions with Dr. Mishra. This laboratory is carrying out its responsibilities under quite difficult circumstances. It has to import laboratory materials such as chemicals and media for vaccine production either from or through India at considerable expense. There are very great difficulties with trained manpower due partly to the need to staff the District Laboratories and Hospitals. Nevertheless the Central Laboratory is professionally managed. It produces vaccines against Newcastle Disease Virus, Haemorraghic Septicaemia, Blackwater Fever and Rinderpest. The fermentation plant was most impressive with 6 large fermentors, a generator, freeze driers and a steam boiler. The Animal Tissue Culture Laboratory, which had been established inside an older facility, was beautifully maintained under what were obviously not ideal circumstances. An excellent discussion on the heat stable recombinant Rinderpest vaccine was most instructive. This laboratory will have an key role to play in bringing in these new vaccines. It will also be involved in using the new DNA probes and the PCR reaction in veterinary diagnosis. This laboratory is being run as well as it possibly could be

under the present circumstances. However the way must be prepared for the new technologies by the provision of adequate resources for the present range of laboratories in microbiology, virology, immunology and biochemistry.

RECOMMENDATIONS

I refer to a number of levels at which Nepal should adopt policies to ensure that it is able to benefit from GEB. At this stage, which is early in the process, my first recommendations involve the education and training of people. There will be no GEB in Nepal without trained staff.

University teaching.

"The universities should revise the biology curriculum"

There are sound general reasons for all universities to review the teaching of biology. The field has changed dramatically since the elucidation of the Central Dogma in the 1960s, but in spite of this there are universities all over the world which still teach biology as if nothing had happened. I am sure from waht I was able to learn that this applies to Nepal.

This is especially relevant to GEB. GEB is a group of technologies which depend heavily on newly acquired knowledge in molecular genetics, microbiology and biochemistry and to a lesser extent in botany and zoology. I strongly recommend that steps be taken to review the biology curriculum in the universities in Nepal to ensure that it will be radically changed to incorporate sufficient material from molecular genetics, microbiology and biochemistry. From what conversations I had I became concerned that the university colleges in Nepal were not in a position to teach students to a sufficiently advanced level in these subjects.

Training.

"Four scientists should be sent abroad for training in GEB"

GEB can only be undertaken by teams of scientists with postgraduate experimental experience in relevant fields. Nepal has only a small number of well-trained research scientists, and so each has a responsibility to cover a much greater range of knowledge than would be usual in a developed country. Even though the demands on staff are great, the Government through RONAST should nominate a small number who should be asked to concentrate on the field of GEB. At this stage in the development of the country I suggest that three people should be sent

abroad for training. UNIDO should be asked to arrange training programmes for 4 researchers one each in:

microbiol diagnostics for the biofertiliser programme;
plant genetic engineering to work on the production of genetically engineered potatoes;
molecular biology of citrus greening disease;
micropropagation as applied to reafforestation.

Research

"The Government should give to RONAST the responsibility for establishing a single national GEB laboratory which would be staffed by scientists from different institutions. This laboratory should have well-equipped and funded laboratories in (i) biochemistry, (ii), microbiology, (iii) molecular genetics and (iv) fermentation".

"The RONAST GEB Laboratory should be associated with the International Centre for Genetic Engineering and Biotechnology".

"A National Committee for GEB should be established with representatives from RONAST, the Central Animal Disease Investigation and Research Laboratory, National Agricultural Research and Service Centre, the National Herbarium and Botanical Gardens, and the Teaching Hospital."

"The National GEB Committee should ask UNIDO for assistance in drawing up a GEB plan for Nepal. A draft plan should be prepared which emphasises training in the context of a small number of research projects which are related to appropriate economic and social needs of Nepal. UNIDO should be asked to send a mission of 6 scientists with appropriate experience to discuss this draft plan with the National GEB Committee, with a view to preparing it for submission to UNIDO for support"

"The RONAST GEB Laboratory should begin as soon as possible to carry out a small number of research projects with the objective of building experience in GEB and of helping to solve certain important social and economic problems"

"The staff of the National Laboratory for GEB should be required to teach advanced courses to a highly selected small group of university students who would be specialising in GEB, probably at Master's degree level."

"These students should be sent for further training to the ICGEB or to other laboratories in countries with strong experience in GEB."

David McConnell. 24 April 1990

APPENDIX I

Names and addresses of persons with whom matters in this report were discussed.

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