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DEVELOPMENT OF A NATIONAL CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY IN THAILAND

U.S. ADVISORY GROUP VISITS TO THAILAND
July 23–August 3, 1984

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NOTICE: The project that is the subject of this discussion paper was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the advisory group responsible for the report were chosen for their special competences and with regard for appropriate balance. However, the material they have provided for this paper represents their individual assessment and recommendations.

This discussion paper has been reviewed by a group other than the authors according to the procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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INTRODUCTION

Thailand possesses relatively well-developed scientific and technical institutions and personnel, but its continued economic and social development will rely heavily on the creative use of its natural and human resources. Economic development is occurring primarily in Bangkok and a few other major urban areas, while the rest of the country lags behind.

Consequently, Thailand's five-year economic development plan emphasizes the need to apply science and technology to increase productive activities throughout the country. Given this emphasis and an agency-wide focus on increased investment in science and technology, the U.S. Agency for International Development (USAID) Mission in Thailand proposes to assist in the development of a new national science program. USAID has asked the National Research Council (NRC) through its Board on Science and Technology for International Development (BOSTID) to join with it and the Royal Government of Thailand (RGT) in implementing such a program.

As a first step, a workshop was organized in June 1984 by the Thai Ministry of Science, Technology, and Energy (MOSTE) and BOSTID to provide input for the development of a strategy for a national program in science and technology for development. The areas of focus at this workshop, which was convened in Bangkok, included (1) bioscience and biotechnology, (2) metallurgy and materials technology, and (3) applied computer and electronic technologies.

Based on this workshop, the Royal Thai Government decided that the initial activity under the USAID program would focus on the development of biotechnology largely because

- Thailand is a fertile country with a vast pool of underutilized natural bioresources that can be developed into useful value-added products.
- Thailand has a relatively large number of qualified professional personnel in the basic life sciences, animal and human health, and agriculture.
- Genetic engineering and biotechnology require relatively little capital compared with other areas of technology, thus increasing their suitability for transfer to Thailand as well as their development locally.

- o The significant progress already achieved in biotechnology can be translated into major improvements in agricultural and industrial productivity and health.
- o Thailand's agricultural yields need to be enhanced by improving disease-resistant crop varieties, controlling mycotoxin contamination, improving milk production, and controlling fish and animal diseases.

Problems related to public health, agriculture, and a rapidly expanding agro-industrial sector make it desirable to utilize various levels of biotechnology. Furthermore, the urgency of these problems does not allow time for traditional approaches nor do their complexities lend themselves to solutions through conventional research alone.

To strengthen Thailand's capabilities in this area, the Royal Thai Government recently established a National Center for Genetic Engineering and Biotechnology (NCGEB). This center is conceived as a national program or a "center without walls" to support research and development, including the coordination and facilitation of communication among the numerous disciplines, in the agricultural and industrial implementation of biotechnology in Thailand.

After the decision was made to develop the NCGEB, BOSTID was asked to arrange a visit by an advisory group to Thailand to make recommendations on the overall establishment of the center, alternatives for rapid and effective commercial utilization of technology emerging from the center, and problems already identified by senior officials of the Royal Thai Government as areas that might benefit from the application of biotechnology. Topics to be considered were discussed in working groups composed of seven United States members and Thai scientists and governments officials. These topics might include:

- o Diseases of cultured freshwater fish
- o Control of aflatoxin in corn
- o Utilization of cassava
- o Development of animal biotechnology
- o Plant cell culture and alternative crops

Reports of the individual working groups on these five areas comprise Part II of this report, while Part I deals with the program of the center and ways to encourage commercial use of its output. It is recognized, however, that biotechnology can make a major contribution to other problems, and it is assumed that they will be addressed as the program progresses. Members of the advisory and working groups are listed in Appendix A.

This report was prepared by Rose Bannigan of the BOSTID staff, based on papers written by Thai participants and U.S. experts. Appendixes B and C were prepared by Yongyuth Yuthavong, Vice-Director, National Center for Genetic Engineering and Biotechnology. The papers have been edited to eliminate duplication, but the final draft was reviewed and approved by the U.S. experts and the Thai organization committee. Sabra Bissette Ledent edited the report.

PART I

OVERVIEW

OVERVIEW

The establishment of a National Center for Genetic Engineering and Biotechnology (NCGEB) in Thailand presents unique challenges, probably unparalleled in the world. The opportunities to integrate a variety of disciplines as well as both the old and new technologies associated with biotechnology and to direct them toward major economic problems confronting Thailand are not in themselves unique. What is unique, however, is the conviction of the Ministry of Science, Technology, and Energy (MOSTE) and of the Royal Thai Government that converting the technological fruits of center research into economic realities will benefit Thailand.

In developing a biotechnology center, the challenge faced is how to formulate a structure that provides sufficient flexibility for the introduction of new ideas and approaches while at the same time maintaining the structure necessary to support national and international credibility for the center. Successful integration of the technologies generated by academia and industry with the government's strong desire to exploit emerging technologies to solve problems and establish new job opportunities could result in a center that even serves as a model for developed countries.

DEFINITION OF BIOTECHNOLOGY

The term "biotechnology" means use of a biological system to produce a product, use of a biological system as a product, or use of the techniques of biotechnology to indirectly provide a product, process, or service. By this definition, biotechnology is an old and well-established technology, which has generated many products, processes, and services.

There are many examples of cases in which a biological system is a product. The most significant examples are crop seeds, including hybrid seeds; animal breeding stock; semen for artificial insemination; and embryos for transplant in animal husbandry. Clearly, biotechnology cuts across many areas.

CONCEPTUAL FRAMEWORK

Biotechnology represents new methodologies that can address some traditional and new problems of Thailand. Fortunately, Thailand has invested in the development of a human resource base in this area at several institutions (see Appendix B). Thus, the new center will help focus these resources on the social and economic development of Thailand, utilizing close linkages between the public and private sectors. Through this process, it will create a critical mass under which new technologies and resources from outside Thailand can be exploited.

ORGANIZATIONAL COMPONENTS

The proposed organizational structure of the new biotechnology center is shown in Figure 1. The Policy Advisory Committee establishes policy and overall direction as well as national priorities and reviews budgets and staff to ensure that national objectives are being met. The International Steering, Oversight, and Evaluation Committee (ISOEC) ensures that international inputs--primarily technical--are introduced into the program at the outset. Through an efficient monitoring program, ISOEC evaluates the progress of all major projects against their specific goals and objectives so as to establish a high level of international credibility.

The center, which in effect coordinates and promotes biotechnology, clearly requires extremely effective lines of communication, not only among its internal bodies, e.g., Policy Advisory Committee and the technical committee(s), but also, and most importantly, with the highest levels of government, such as the ministries of science, technology, and energy; industry; health; and agriculture and cooperatives. It will also link the universities at both the rector level, through its Policy Advisory Committee, and at the working level, through its technical committee(s).

International linkages are essential to ensure recognition of the center worldwide. At the outset, international representatives should sit on the center's International Steering, Oversight, and Evaluation Committee, which will participate in the review process. The international stature of the center will depend upon the credibility of the science that emerges from its program. This credibility will be facilitated by the participation of Thai scientists in international and regional scientific meetings and symposia. Such participation should be encouraged and supported.

Policy

Statements governing policy of the new center must be sufficiently flexible to allow individual judgment at the lowest possible levels, while providing a framework of guidelines that constitute an appropriate organizational structure. Several general guiding principles are essential:

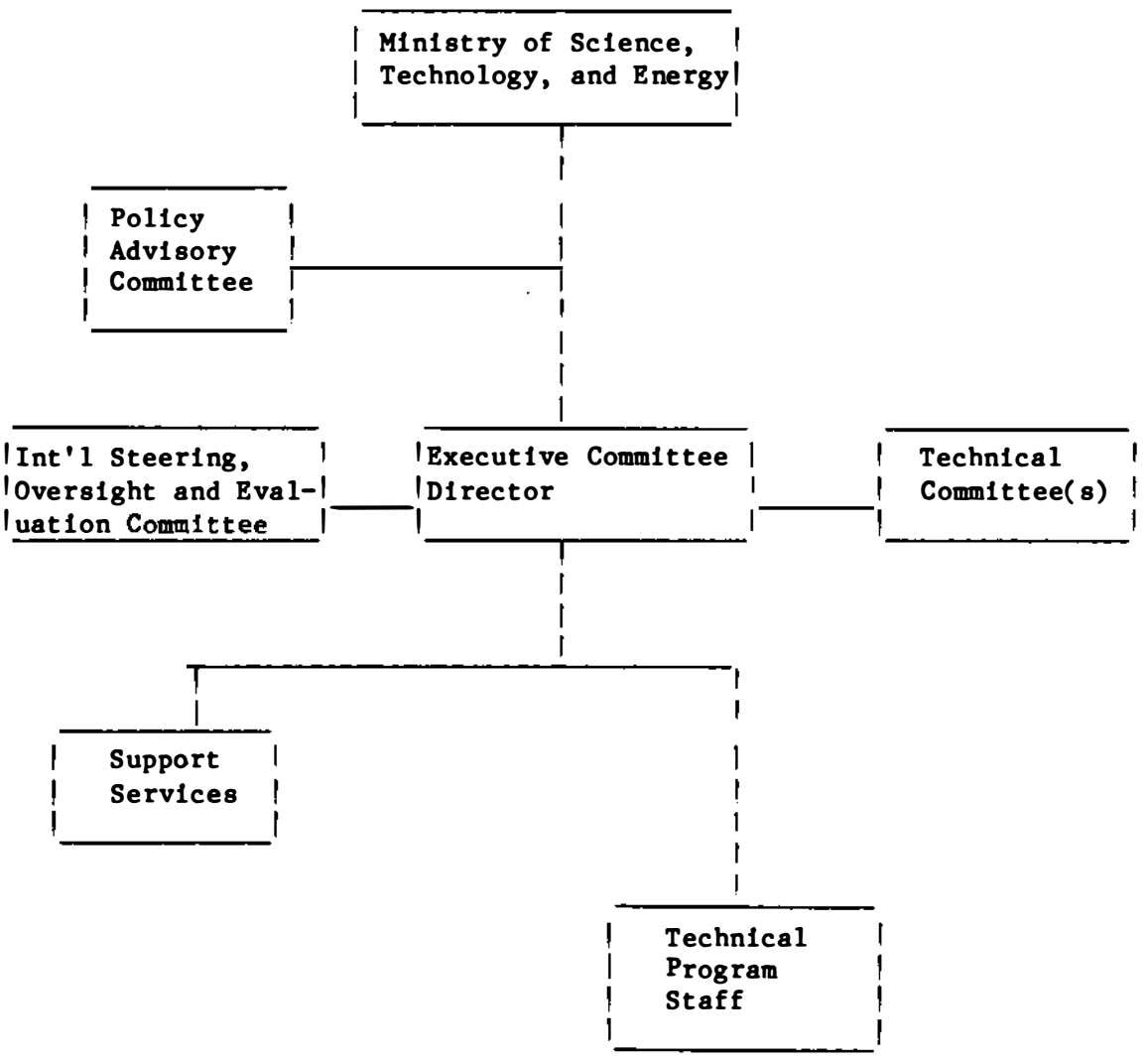

FIGURE 1 Proposed organizational structure of the National Center for Genetic Engineering and Biotechnology.

- o There must be balanced representation within the center, including
  - -- various ministries at the policy level,
  - -- the private sector at both the technical and policy levels, and
  - -- all participating universities.
- o Research programs must be developed based on clearly defined and established goals and objectives, and implemented on the basis of merit. Once a program is initiated, its progress toward goals should be monitored throughout the life of the program. Open-ended research without objectives and time-defined goals should not be encouraged.

Industrial representation on center committees should occur at several levels:

- o Policy. Questions of patent exclusivity, royalty rights, and coownership of inventions should be resolved within the Policy Advisory Committee. Representation from the private sector should be at senior levels of people responsible for both the commercial and technical aspects of the business.
- o ISOEC. The industrial representative should be a senior, established technical person with a sound technological background, but not necessarily scientifically active.
- o Technical committees. At least two individuals from the private sector should be on each committee. One should be strong in the technical and scientific areas, and the other should have strengths in engineering and the commercial aspects.

Both short- and long-term research programs should be conducted. The former must contain clear statements of potential economic impact and inferences as to how the new technology can be utilized.

Patents

Provisions must be made for patent of novel discoveries generated by the research center programs. Efforts to obtain patents should be encouraged. Once a patent position has been established, industry should be allowed to bid for an exclusive position with the patent. Any modifications, e.g., when coexclusivity has been determined more beneficient, should be agreed to at the policy level. Royalty payments, length of exclusivity, etc. would be established on a case-by-case basis with final approval residing with the Policy Advisory Committee.

Sources of Funding

Although funds should be sought from a variety of sources, including the private sector, the Thai government (via grants or loans from the United States or other countries) should remain the dominant supporter. The government should always maintain an adequate level of core support to retain basic control over the program so that the original objectives of the center are not compromised by relying solely on outside funding.

STRATEGIC ASPECTS

Balance

Balance throughout the structure of the center is essential to its success. This need reflects the rather ubiquitous nature of biotechnology and the vast array of opportunities exploitable utilizing biotechnological

techniques. Balance must start at the top and include ministry representatives in industry, health, and agriculture as well as science, technology, and energy, as these ministries will unquestionably participate in the development and use the products of the science itself. Furthermore, balanced representation of the various universities on the technical committee(s) and the Policy Advisory Committee must reflect the participants' individual areas of expertise in health, agriculture, and engineering.

Self-Evaluation

A functional evaluation program conducted through an outside review process is needed to structure and maintain a center that has credibility and attracts international attention. This program should include both scientific review as well as review of the potential technological impacts, e.g., through industrial representation.

Resource Deployment

The vast majority of researchers will, of course, be housed at their respective universities and will use their own equipment. Although the mechanism for capital purchases will be resolved later, it is essential that revenue expenditures be sorted out as soon as possible as funds are already being spent.

Technology Transfer

The Ministry of Science, Technology, and Energy and its current minister have made a major commitment to full utilization of technologies produced by the center. The minister has clearly stated that the transfer process must enhance the economic viability of Thailand through the generation of new industry and opportunities. This process can be partially assured by policy statements, industrial representation in the program aspects, and provision of proper patent and license protection. Only continual monitoring, however, will ensure full utilization of research.

Commercial exploitation of technology generated by the center is the primary objective, but it is envisaged that much additional and useful information will be produced. Publication of this information in scientific journals will be strongly encouraged through the technical committee(s), and it will be carried to the public through the existing or new extension services and other means of communications.

In addition to ensuring that the technology developed is conveyed to the private sector, the center should also be able to advise and serve members of the private sector upon request regarding existing biotechnological processes. This is especially needed at the current time as small- and medium-sized industries do not have a research and development capability, and they are seeking the appropiate technologies

and processes from abroad. For these cases, the center could use individual consultants as well as multidisciplinary, interinstitutional teams to offer guidelines and services to a company or ministry considering purchase or utilization of an existing technique or equipment. Provision of these types of services, however, requires that the center have the ability to sign secrecy agreements with private companies, especially when the latter are paying for the services. This will increase the value of the center to the private sector in the biotechnology field and pave the way for a strong industrial-biotechnology association. Further elaboration regarding implementation of such an activity will require greater in-depth study.

OPERATIONAL ASPECTS

Considerable resources have been invested in the development of a biotechnology capability in Thailand. A large number of young professionals have been trained in the country and at foreign universities and institutions. Modern instrumentation and, in some cases, support facilities have been developed in several laboratories. Finally, senior universities are in various stages of institutionalizing biotechnology programs (see Appendix C), with the result that Thailand has attracted international attention to this field of research. Indeed, some of the activities may contribute to the solution of problems in other countries.

The Royal Thai Government has recognized that successes in agriculture and the development of large food surpluses for export are not enough to continue to sustain a rapid rate of economic growth. Quality control is extremely important. Priority has been placed on industrialization, and particularly those types of industries that depend on comparative advantages in human resources or raw materials. Biotechnology has been recognized as one area in which the universities should assist in economic development through support of the private sector.

Nevertheless, several constraints must be recognized if the universities are to play a major role in nontraditional areas of economic development. Historically, many public educational institutions have been wary of overinvolvement in national economic development and what they perceive as a possible erosion of more traditional types of intellectual activities. Academics are also aware that the public might perceive selfish motives in their involvement in activities that stress economic goals.

Formal relationships with the private sector should nevertheless be encouraged. The academic structure in Thailand promotes the independence of the professor in his research goals rather than encourages mission orientation and problem solving. Procedures for performance evaluation, the measurement of progress toward research goals, and a corresponding reward system should be fully elucidated. These procedures should also address the applied aspects of the researcher's work and relationships to the industrial development of Thailand. Based on these considerations, it is recommended that the biotechnology program

be structured to address institutional issues in addition to research topics so that university research is more applied and oriented toward the improved economic and social development of Thailand.

The biotechnology program should include two types of grants. Capacity-building grants to institutions would focus on improving their capability to carry out research on specific problems. In this way, activities are oriented toward the public good and have high potential for payoff. Individual grants would be competitive and responsive to proposals that are clearly innovative, represent good science, and show promise of significant benefits to the country. It is also recommended that an international steering, oversight, and evaluation committee be appointed to coordinate the grant-making process.

International Planning, Oversight, and Evaluation Committee

It is recommended that an external committee be appointed that would include Thai and foreign scientists not directly involved in the concerned institutions or projects. Membership might include well-known scientists, research directors, and other administrators from either the public or private sector. The committee would make recommendations to the NCGEB.

The committee should be responsible for developing specific guidelines for the grants (capacity building and individual) and for reviewing and approving all capacity-building grants. As a "friend of the program," it would work with institutions or individuals to aid in the formulation or improvement of promising submissions where help is needed. Inputs of the committee could include assistance in promoting linkages to other domestic or foreign institutions, projects, and the private sector, where such relations would be truly beneficial. Its responsibilities to oversee and evaluate progress and products would continue throughout the lifetime of the project. This group would enhance the credibility of the program and serve as a catalyst for improving collaboration, international recognition, and financial support, and it would ensure that high standards of research quality are maintained.

Capacity-Building Grants

The primary purpose of the capacity-building grant is to build a self-sustaining capability in Thai universities for addressing problems of national importance and amenable to a biotechnological approach. Capacity-building grants would be awarded to research groups solving problems related to agriculture, environment, energy, public health, and industry. Awards would be given on the basis of comprehensive proposals from institutions, and would include the following criteria:

- Projects with potential to contribute to the economic and social goals of Thailand.

- o Evidence of institutional machinery that could develop, encourage, and support the projects.
- o An institutional process and procedure for defining, selecting, monitoring, and evaluating projects, as well as a mechanism for monitoring and evaluating faculty and project performance.
- o An advisory committee with representatives from the private and public sectors to enhance interaction with outside groups, aid in problem definition, and promote utilization of research results.
- o Ties to other public and private entities.
- o Accountability for all of the above.

Capacity-building awards would absorb approximately one-half of the grants budget. At the outset, it should be recognized that not all institutions may be awarded grants, especially if their proposals are not appropriate and hence not approved, or that some institutions may be delayed in a formal start for other reasons.

Individual Grants

Within academic organizations, opportunities should always exist to accommodate creativity and innovation. Administrative structures exist to facilitate an institution's assigned role but may not encourage intellectual independence. Occasionally, a scientist may have new insights or an imaginative new approach that can significantly advance his or her field and result in long-term solutions to the problems of society. This type of activity should also be nurtured if a program is to grow beyond predicted bounds. Therefore, it is recommended that up to one-quarter of the grants budget be used in a competitive grants program to encourage innovation and imaginative research methodology. (The remaining one quarter of the budget will be used for other program costs.)

Proposals would be submitted in an approved format (see Appendix D). The description of research should include rationale, linkages, anticipated benefits, realistic schedule, and institutional responsibilities. Proposals should be especially encouraged in the following areas:

- o Agriculture and energy
  - -- Food production and quality
  - -- Use of by-products, e.g., rennin
  - -- Improvement and conservation of germ plasm
  - -- Postharvest processing
  - -- Development of new marketable products
  - -- Biological processes that reduce fossil fuel inputs
  - -- Biological pest control
  - -- Milk and meat production
  - -- Reproduction of organisms
- o Health and Environment
  - -- Vaccine development
  - -- Diagnostic procedures and agents

  - -- Drugs from plants and microbial sources
  - -- Genetic abnormalities
  - -- Animal and human disease control
  - -- Nutritional biochemicals
  - -- Toxic residues in agricultural products
- o Industrial
  - -- Products with a commercial value
  - -- Analytical procedures
  - -- Improved efficiencies in processes
  - -- Waste utilization
  - -- Pilot plants
  - -- Enzyme production and utilization
  - -- Industrial development of agricultural products, e.g., rubber.

Individual grants would be awarded through an institution, but the funds provided would be expended by the selected investigator. Because the grant would be associated with an individual, it would be reconsidered if his or her affiliation with the institution terminated.

SUPPORT SERVICES

Thailand has developed a relatively large cadre of well-trained biotechnologists. The described program for institutionalizing biotechnology will permit these human resources to be used more effectively in addressing the social and economic goals of the country. The addition of support services to strengthen the national capability is also necessary. Information acquisition, communication, and training are all components of a technical support system.

Information Acquisition and Dissemination

It is imperative that the new center have funds available to establish access to the scientific and technical information contained in international or other national repositories and to cover subscription and user fees. At the present time, vast amounts of data can be easily accessed by computer through on-line searches of national and international data bases. While acquisition of this technology represents a substantial financial investment, it is a vital source of information to researchers about to undertake problem-oriented research. Furthermore, once established it is a highly cost-effective means of obtaining information on research compared with the traditional means of text, journal, and abstract acquisition, the costs of which have increased greatly. The immediate accessibility of the information through computer networks is a further advantage. Finally, this capability will permit communication between research groups at different institutions and the center, as well as circulation of information among research workers and coordination of research activities.

Acquisition of this capability will require funds for the purchase of the computer hardware (modems) and software and subscriptions to data bases, as well as funds to meet the costs of data transmission. Initially, it may be necessary to help MOSTE work with the Department of Post, Telephone, and Telegraph (DPTT) to facilitate adequate telephone connections among the various critical cells and with international data bases.

In addition to employing this capability for its own internal needs, the center could serve as a critical resource to the private sector in its search for data on biotechnologies. The requesting company could be charged the actual cost of the service.

The center could also participate in computer conferences with researchers in other countries working on similar problems. It is important that the research results obtained be circulated to other national or international data bases for acknowledgment of Thailand's contribution to problem-oriented research in the areas of bioscience and biotechnology.

National Data Bank

It is essential that a national data bank on bioscience and biotechnology be established at the center to facilitate communications between research personnel within institutions and between institutions in adjacent fields, as well as to acquaint research staff with international science and technology advances. Researchers should have access to this repository or technical data bank when preparing proposals.

Conferences, Symposia, and Workshops

Conferences, symposia, and workshops are indispensible mechanisms for researchers who wish to advance their capabilities and understanding by discussing research results and identifying breakthroughs in biotechnology. These should include both meetings within Thailand to permit interaction between all scientists involved in biotechnological research and activities outside of Thailand.

Publications: A National Journal and a Newsletter

A national journal focusing on research results obtained by Thai scientists could serve as an important link between Thai scientists and their colleagues abroad. A newsletter, as opposed to the more formal documented research papers prepared by Thai scientists funded by the center, would permit the center to share more informal information with a wider audience and to outline information on the activities and problem focus of the center. Such a newsletter could describe projects underway, scientists involved in the projects, as well as their location. It could also serve as a way of communicating with scientific

groups in other parts of the educational system, including undergraduate groups and possibly secondary schools, as well as with the private sector.

National Germ Plasm Collections

Collections of genetic material already exist in Thailand for agricultural, health, and other applications, but these facilities need to be strengthened and expanded in light of the new research, development, and commercial applications with which the center is concerned. This support will not only enhance the capacity of these existing collections, but will also increase the availability and accessibility of organisms to scientists and in support of commercial enterprises.

Training and Retraining Plan

A major driving force behind the biotechnology program is the hope that biotechnology will provide solutions to major economic problems confronting Thailand. A second and perhaps equally important force is the need to increase and maintain a well-trained cadre of professionals in biotechnology. The educational aspects of the program have a high probability of success, and this may be even more important than the primary motive for the research.

Retraining is an extremely important factor within the educational program. It becomes even more significant in a country having a limited number of professional people. Scientists affected by technical obsolescence need the opportunity to develop a new area of expertise.

Networking

Although the extensive networking required among various universities and the center is described elsewhere, there has been no mention of the extensive networking in the form of information exchange required with other groups, both national and international, outside the center. These include professional and user groups.

Networking with industry would occur on an individual basis, particularly if there is opportunity for an exploitation step. General principles and nonspecific areas of general interest of the center's work would go through the industrial representatives of either the technical or advisory committees.

PART II

WORKING GROUP REPORTS

WORKING GROUP REPORTS
1. DISEASES OF CULTURED FRESHWATER FISH IN THAILAND

INTRODUCTION

This report provides information about the diseases of cultured freshwater fish in Thailand and identifies specific research goals designed to solve the major disease problems limiting production. Species of principal concern are the sand goby (Oxyelectris marmortus), snake head (Ophicephalus striatus), and walking catfish (Claris sp.). Modern techniques for improved detection, prevention, and control of disease among the population of cultured fish has been enhanced by recent advances in biotechnology. In Thailand, there is a need to establish the diseases of importance and their causative agents and to provide mechanisms for their control.

Disease clearly plays an important role in the production of fish in Thailand. A classic example is an ulcerative lesion of the three species of fish indicated. This condition was first observed in the south of Thailand in 1982 and has since spread to all freshwater fish-rearing areas of the country. In addition, it is now known to occur among populations of fish in Laos and Kampuchea and may have extended into Burma. The economic losses in Thailand resulting from this one disease in 1982-1983 were calculated to be 105 million baht (U.S. $4.5 million). The economic loss for the following year (1983-1984) could not be estimated because of flooding. The cause of this ulcerative disease remains uncertain and should become the subject of intensive investigation.

PROJECTS

PROJECT: Causes and Control of an Ulcerative Disease Among Economically Important Cultured Fish of Thailand

This investigation would incorporate bacteriology, mycology, parasitology, virology, histology, immunology, and toxicology in the study of the cause and control of ulcerative disease.

Statement of the Ulcerative Disease Problem

Since October 1982, mortality among cultured fish of several species has resulted in economic losses approximating U.S. $4.5 million. Species of primary concern are the snake head, sand goby, and catfish. Although bacterial, fungal, parasitic, and viral agents have been isolated or observed associated with diseased fish, none of these are considered the primary cause. The three cultured species of fish exhibit similar pathology, initially characterized by the appearance of raised areas of induration and erythema. The subsequent erosion of these areas results in the formation of an ulcerative lesion on the body surface. Ulcers characterizing the condition may appear on any area of the body, but were often noted in the head and eyes, resulting in blindness and, eventually, death.

The appearance of these fish make them unacceptable for marketing since buyers are reluctant to purchase abnormal animals. This is understandable because the consumption of diseased fish is undesirable, particularly when the causes of the condition are unknown. This has had a direct impact on the availability of domestically produced quality fish protein. In addition, it represents a loss of revenue generated by those species usually used as export products.

Present Understanding of the Problem

The following observations of ulcerative disease were compiled by the working group on freshwater fish diseases of Thailand:

- Occurrence of the disease seems to be independent of water temperature; however, this has not been tested under experimental conditions.
- The appearance of the disease is dependent on and occurs with the onset of the rainy season (October through March).
- The disease occurs in both cultured and wild populations of fish. It is estimated that during certain times all wild fish may be affected.
- The disease occurs among cultured fish when water from flooded agricultural lands on the rivers and canals is allowed to enter the pond.
- Lesions may appear as soon as 24-72 hours after water from canals or rivers enters ponds containing fish.
- One way of diminishing losses due to the disease is to prevent introduction of water into the ponds. However, this step results in a deterioration of water quality that requires constant surveillance and management to insure successful production. These techniques are both costly and time-consuming.
- All three species are subjected to very high-density cultural conditions, making more imperative the need for improved environment and water quality.
- When diseased fish are moved to improved environmental conditions, significant recovery occurs.

- o If diseased fish are placed with unaffected animals under laboratory conditions, there is no transmission to the healthy animals.
- o No single parasite seems to be responsible for the disease, although bacterial, fungal, parasitic, and viral agents have been isolated or observed in association with the lesions.
- o Environmental factors seem to be predisposing the fish to subsequent attack by microorganisms that are normal pond inhabitants.

Table 1 indicates disease agents which have been observed in the presence of fish with ulcerative disease.

RECOMMENDATIONS

A research project should be initiated addressing the three major areas listed below in order of importance.

- o Water quality
- o Toxicology
- o Infectious diseases: bacteria, virus, protozoa, and mycotic agents

One component of this proposal should be possible development of a research center, by the National Inland Fisheries Institute, concerning disease for fish coordinated. This center would address the following problems: water quality, toxicology, and infectious diseases.

Water Quality

- o Detect compounds prevalent in the water that may cause subsequent disease in cultured fish. This could be accomplished through a program monitoring the sites that suffer from the disease as well as areas where it does not occur.
- o Identify compounds associated with the onset of disease.

Toxicology

- o Examine the effects of individual compounds at acute and chronic levels on fish under laboratory and field conditions.
- o Examine the synergistic or antagonistic effects of mixtures of these compounds on fish health.
- o Examine the residues of such compounds in fish used for human consumption.

TABLE 1 Disease Agents Observed in Association with Freshwater Fish Showing Signs of Ulcerative Pathology

Fungi	Protozoa					
	Ciliata	Flagellata	Myxosporida	Suctoria	Monogenetic Trematodes	Copepod
Saprolegnia	Chilodonella	Costia	Henneguya	Acineta	Dactylogyrus	Lernaea
Unidentified fungus	Ichthyophthirius Trichodina		Myxobolus Thelohanellus	Epistylus Glossatella Scyphidia Zoothamnium	Gyrodactylus	

Bacteria Isolated

Flavobacterium sp.	Aeromonas hydrophila	Pseudomonas fluorescens	Vibrio parahaemolyticus
Edwardsiella tarda		Streptococcus sp.	Pseudomonas sp.

Virus Isolated

Infectious pancreatic necrosis virus similar to Ab serotype

Infectious Diseases

- Examine the predisposing effect of the compounds found in the water on subsequent invasion by infectious disease agents.

This is an important problem requiring attention by a team of scientists such as those recommended. Fish disease may be an indicator of the general degradation of the environment through the indiscriminate use of pesticides and antimicrobial compounds on and in the lands and waters of this country. It would be a mistake not to consider the possibility that a major public health problem has been created.

GENERAL KNOWLEDGE

Worldwide

Since 1945, more information has become available on diseases of fish, the causative agents, and means of control. New technology made available in recent years has been very helpful in understanding the host-parasite relationship, immunity, the role of the carrier, virulence, and disease control. Human and animal health have improved as a result of advances in understanding the disease process.

Thailand

Thai scientists have an active research and diagnostic program in the fish health field. They are well aware of the problems of disease in both cultured and wild fish. Little is known anywhere that will be of immediate or long-term assistance with ulcerative disease.

CAPABILITY

Human Resources

Thai scientists working on diseases of fish are well trained in bacteriology, mycology, and parasitology. They need training in virology, tissue culture, and modern cellular immunology.

Facilities

Excellent facilities are available for bacteriology, mycology, and parasitology. Work on viral diseases of fish will require installation of specialized equipment for both fish tissue culture and virology. Some immunology equipment is required, but it is minor compared to that needed for cell culture and virus diagnostics and research. A reallocation or redistribution of space may be required to meet the future needs of the fish disease group.

CONSTRAINTS

In view of the rapid expansion of the disease problem, the capabilities of disease scientists may be limited. Advanced knowledge and technology are required to solve disease problems, and it is extremely important that staff be trained (or retrained) in virology and immunology. Equipment not available in the laboratory for virus research is a limiting factor.

TIME REQUIRED TO SOLVE THE PROBLEM

The large geographic area where the disease is known to occur and the fact that it is in both wild and cultured fish creates an added difficulty in estimating the time required to solve the problem. It would be best to conduct an intensive three-year study followed by an in-depth review of the results before extending all or any part of the research for an additional period. The disease process among fish in Thailand is very complex and may involve more than just the common host-pathogen interaction.

PROBABILITY OF SUCCESS

It is not known if these problems can be completely solved, but it is certain that a program in diseases of cultured fish can improve production. At present, production has been curtailed because of concern over this nontreatable disease.

This program could also bring about important technical advances in fish culture and management in Thailand. It will no doubt advance the science of fish health from which all will benefit.

ALTERNATIVES TO SOLVING THE PROBLEM

One short-term alternative would be the introduction of new species of fish for cultivation that are more resistant to conditions in Thailand. However, one must avoid the introduction of exotic pathogens with new fish species brought into Thailand for cultivation.

Over the long term, one might try some selective breeding experiments to develop resistant stocks. It should be noted, however, that this has not worked well with other species of fish and disease agents.

BIOTECHNOLOGY AND ITS ROLE IN THE CONTROL OF INFECTIOUS DISEASES OF CULTURED FISH IN THAILAND

Initial studies must relate to the cause of the disease and methods of management for partial control. This information will allow the application of new and improved techniques derived from biotechnology

to be instituted for detection, prevention, and control of infectious diseases.

Vaccine Development

Immunotherapy is by far the most efficient means of maintaining the long-term health and disease resistance of important fish stocks. It is important that the development of prototype vaccines be undertaken with proper testing in vivo. Areas of study should include:

- o Isolation production and testing of whole-cell vaccines
- o Isolation and purification of important immunogenic antigens from pathogens
- o Chemical engineering of immunopotentiated vaccine material
- o Investigation of optimal routes of immunization
- o Cloning of genes for the protective antigens of viruses and parasites.

Assessment of Immunocompetency

The development of standard immunological assays is essential if fish populations are to be successfully monitored for their ability to respond to immunological insult. Such analytical tools allow investigators to diagnose harmful trends which may lead to decreased resistance to disease. These assays may be applied to the study of wild and hatchery-reared animals, animals exposed to crowding stress, toxicants, pollutants, various diets, or any other parameter possibly affecting the health of fish stocks. The studies described below would assess the function of the B-cell, T-cell, and phagocytic components of the immune response, as well as resistance of the animals to pathogenic insult.

Immunological Assessment Studies

These should include:

- o Serum antibody responses to highly immunogenic bacterial antigens (should be developed with an appropriate assay system)
- o Antibody-producing cell responses to standard bacterial antigens
- o Proliferative responses of lymphocytes to standard antigens or mitogens
- o Phagocytic responses to standard antigens
- o Response to standardized challenges of lethal pathogens

Diagnostics

The development of monoclonal antibody techniques suitable for use in the fish-pathogen system would be very useful. These methods have

already been tested with certain species of fish; however, this work has just begun. Improved methods of cell and organ culture in animals may also be applied to the cultured species of fish in Thailand. This could be very important in virus isolation, study of pathogens, and vaccine development.

2. CONTROL OF AFLATOXIN IN THAI CORN

INTRODUCTION

Prior to 1971, aflatoxin contamination of corn (Zea mays L.) was considered primarily a postharvest storage problem. Subsequent extensive research (Diener et al., 1983), however, has demonstrated that aflatoxin contamination of corn in the field before harvest is a serious problem in the southeastern United States. Corn is a major ingredient of swine, poultry, and cattle feed. Animal losses from aflatoxin in corn, causing mortality and subtle losses in weight gain and feed conversion, have been costly, particularly in certain years (1973, 1977, and 1980). Aflatoxin has also taken human lives in at least two catastrophic incidents, and the epidemiological data point to a direct relationship between the level of aflatoxin in the human diet and cancer (Council for Agricultural Science and Technology, 1979).

The prevention of preharvest contamination of corn by Aspergillus flavus has been a research priority in the United States for 10 years. Control through genetic resistance, pesticides, detoxification, and agronomic and cultural practices has been intensively explored for both corn and peanuts. Although differences in aflatoxin levels in commercial maize varieties have been reported, the inability to repeatedly demonstrate these differences in experiments conducted over several locations and years challenges the concept that aflatoxin formation in corn is under genetic control. Sources and mechanisms of genetic control with a chemical basis for resistance to A. flavus invasion, infection, or aflatoxin production have not been identified. Analyses of samples from over 200 varieties of 20 commercial seed companies grown at 12 locations in Alabama from 1976 to 1981 have resulted in the conclusion that there is no resistant germ plasm among current U.S. varieties.

Pesticides, including insecticides with some fungicidal properties as well as systemic fungicides, have failed to control A. flavus or prevent aflatoxin contamination in field corn. This is possibly because A. flavus is a saprophyte or a weak parasite at best rather than an aggressive plant pathogen and only capable of obtaining nutrition for growth from dead or dying plant tissue or seeds of low physiological activity. Insect injury to kernels, drought stress, and entrance for infection via dying silks are usually related to years of epidemic aflatoxin contamination of maize before harvest in the United States.

Among agronomic practices, the planting date, adapted varieties, optimum plant density, and good cultural practices, such as weed control and fertility balance to alleviate stress during grain filling, are considered useful in reducing preharvest aflatoxin contamination. Preventing drought stress by proper and timely irrigation can prevent and reduce preharvest aflatoxin contamination in both corn and peanuts.

Preventive measures are usually the most economical and effective, but the detoxification of contaminated corn, peanuts, and cottonseed with ammonia in some form has been universally successful for reducing aflatoxin from high to negligible levels.

In Thailand, aflatoxin contamination of corn appears to be most closely associated with the crop harvested in July and August during the rainy season. Data from the Thai Department of Agriculture for the years 1980-1983 indicate that aflatoxin in samples taken from farmer storage houses, middleman storage, and commercial silos ranged from 0 to 750 parts per billion (ppb) (Siriacha et al., 1983). In general, the percentage of positive samples increased with time at all locations. Thus, postharvest contamination by aflatoxin buildup in inadequately dried kernels appears to be the major problem in Thai corn. Preharvest samples showed only 4.6 percent contamination in 130 samples, but it is not clear from the report whether this represents one year's or several years' data. The incidence of levels above 100 ppb was frequent enough to be of serious concern. Frequency of occurrence of A. flavus in corn samples indicates widespread distribution of the fungus in corn.

Data on environmental, agronomic, and management factors that are favorable or depressant to the fungus and aflatoxin production are not available for evaluation. These data are needed to indicate the nature and circumstances of the invasion of Thai corn by the fungus as well as which research on control will be most successful in the long term.

PROJECT

The overall objective of the project is to improve the quality of Thai corn by reducing aflatoxin contamination by Aspergillus flavus, using shortand long-term measures.

Specific objectives are:

- To determine the extent of natural preharvest aflatoxin contamination in Thai corn over at least three seasons.
- To determine the effect of planting date, drought, irrigation, temperature, minimum tillage, and other cultural practices and environmental factors on aflatoxin contamination in Thai corn.
- To determine the conditions, time, and other factors influencing effective detoxification of aflatoxin-contaminated corn by some form of ammonia or by fermentation methods.
- To evaluate the silk inoculation technique with A. flavus spores as a viable method for screening corn varieties for resistance to preharvest contamination.
- To evaluate varieties in which the ear shank bends and the ear points toward the ground after physiological maturity to

determine if these ears have lower seed moisture contents than conventional varieties, and to further determine the time relative to maturity date for harvesting well-adapted local varieties to obtain the lowest seed moisture content--all methods of reducing postharvest aflatoxin contamination.

- o To screen germ plasm with potential aflatoxin resistance (to be collected locally and internationally), crossed to well-adapted local varieties and selected for low fungus invasion and aflatoxin formation in progeny. Testing may be by silk inoculation and other promising techniques.
- o To determine the effectiveness of prompt crib drying in preventing postharvest aflatoxin contamination.

CAPABILITY

Human Resources

The research team could consist of Dr. Sutat Sriwatanapongse (principal investigator), corn breeder, Department of Agronomy, Kasetsart University; Dr. Chamnan Chutkaew, corn breeder, Department of Agronomy; Dr. Supot Faungfupong, agronomist, Department of Agronomy; Dr. Chalermlarb Chuaiprasit, pathologist, Department of Plant Pathology; and Dr. Orapin Bhumibhamon, food technologist, Department of Biotechnology. Dr. Carlos De Leon, plant pathologist, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) could serve as an international collaborator.

This group appears to represent a critical mass of competent scientists interested in aflatoxin research and problem-solving. They have excellent training, and they are young, enthusiastic, and well-regarded by their peers. Cooperation within this group should not be a problem, since they are closely associated at Kasetsart University and hold each other in high regard which is essential to quality team research involving several disciplines. Research may also involve the Department of Agriculture (currently providing aflatoxin analyses) and at least these three departments at Kasetsart University.

Facilities

At present, two laboratories owned by the Department of Agriculture can provide the analyses of aflatoxin. One laboratory is located at the Bangkhen Campus and the other at Prabhutabat Station, Saraburi. It is not convenient for Kasetsart University researchers to use either of these laboratories, since the one at Bangkhen Campus is extremely busy and the laboratory at Prabhutabat Station is too far, thus involving considerable travel time. Moreover, it is difficult to obtain the services and control of the laboratory technicians since they are under the supervision of another organization. On a continuing basis, therefore, Kasetsart University should have its own aflatoxin laboratory.

The Kasetsart University Corn Program has one protein laboratory where analyses for protein and amino acids in corn are run. With the addition of limited equipment, the aflatoxin analyses can be conducted there.

Equipment

CIMMYT has already provided equipment for the Kasetsart University aflatoxin laboratory, including a UV block, hood (without blower), rotary vacuum evaporator, Waring blender, desiccator, plates with silica-gel and spotting pipettes, and other small items. This laboratory can provide aflatoxin analysis only when additional equipment, such as a fluorescence spectrophotometer and photodensitometer, are acquired. (See the Appendix for a list of the required aflatoxin analytical equipment, glassware, chemicals, and supplies.)

Technicians

One chemist with a B.S. degree is in charge of the Kasetsart University laboratory, and one position is vacant. Because this chemist does not have much experience in aflatoxin analysis, training for a month or two will be quite helpful.

Importance of the Aflatoxin Laboratory

Besides undergraduate study, Kasetsart University now has graduate programs (M.S. and Ph.D.) in many fields. The aflatoxin lab will play an important role in generating research activities which in turn will support student training. This laboratory can be used for retraining technicians from other organizations as well.

Biotechnology/Genetic Engineering Laboratory

At present, Kasetsart University has two central laboratories, one at Bangkhen and another at the Kamphaengsaen campus. Each laboratory is equipped with almost all the sophisticated equipment related to biological science and biochemical research. With well-trained and competent scientists (Ph.D. level) in the fields of genetics, animal breeding, plant breeding, and fish breeding and the establishment of biotechnology and genetic engineering laboratories, the fruits of research will appear rapidly. This task could be undertaken by simply utilizing the existing equipment in the central laboratory at Kamphaengsaen and Bangkhen.

Training in the fields of biotechnology and genetic engineering should be emphasized in the first phase of development. Research in the areas of embryo culture, protoplast fusion, plant cell transformation, etc., could be developed during the next phase.

CONSTRAINTS

The current constraints are:

- o Funding for analytical equipment
- o Continuing support for the research staff
- o Continued provision of expendable supplies
- o Training and retraining of research staff

TIME REQUIRED TO SOLVE THE PROBLEM

Aflatoxin is not a new problem internationally. Although extensive research has been conducted in the United States and other countries, the problem still remains. It is doubtful that it will be solved quickly or that aflatoxin contamination will be totally prevented in corn or in any other crop. The economic effects of aflatoxin can be reduced, however, so that losses are minimized at all levels of the Thai corn industry. Short-term solutions, such as ammoniation of contaminated corn, can be verified experimentally as effective for Thai corn with the facilities available. A vigorous three- to five-year research program could develop data to support short-term management solutions, as well as technology for detoxification of contaminated corn. This program could also determine the potential for success of a long-term breeding program for aflatoxin resistance.

PROBABILITY OF SUCCESS

Technical Success

Technology through the acquisition of scientific knowledge of the nature of aflatoxin contamination in corn in Thailand, management, cultural practices, and other approaches will be utilized to reduce aflatoxin contamination. Technology transfer offers an almost immediate solution as contaminated corn can be used as feed after detoxification with ammonia. Because aflatoxin is an international problem, potential sources of resistance in corn varieties can be obtained, new techniques shared, and new technology transferred.

Development of resistant varieties, the most effective and economical approach, requires several years and crop generations once a source of resistance is identified. A biotechnology approach may provide a source of resistance, but it does not represent an immediate solution, since it is probably a long-term technique likely requiring extensive development. Limited preliminary data on corn varieties which at harvest had drooping ears with decreased kernel moisture content seem to offer an innovative, totally new approach for varietal resistance in Thailand. The key to preventing postharvest contamination by fungi is rapid drying to a safe kernel moisture content of 14-15 percent. Thus, if preharvest contamination is not the major mode of aflatoxin contamination in Thai corn, this approach to reducing postharvest losses

is promising for the long term, since a promising new variety must be crossed and back-crossed to local varieties to make an acceptable production variety. For the latter, however, cell fusion may offer assistance.

Commercial Success

Thailand's agricultural products provide 60 percent of the nation's total export earnings and about 25 percent of its gross domestic product. Tapioca, rice, and corn are the major exports. As one of the world's few net food exporters, Thailand could become the future "supermarket" of Asia.

Corn is becoming more important as an export commodity since the advent of marketing problems with tapioca in Europe. It is anticipated that production will increase from 3.5 million to 10 million tons in 10 years without increasing land areas. This can be achieved through the use of modern technology, such as hybrid seed, improved cropping systems, and cultural practices.

Thailand's agriculture has now reached the stage of self-sufficiency and is moving toward commercial industry. Good-quality products are essential to competing in the world market. Thus, a project to improve the quality of Thai corn by reducing aflatoxin contamination is quite timely and appropriate to maintain economic stability. This will also serve as a major guideline in resolving the same problem in other crop commodities.

ALTERNATIVES TO SOLVING THE PROBLEM

Short Term

Detoxifying contaminated corn with ammonia is a short-term solution. Prompt harvest at maturity followed by some form of drying is essential for both long- and short-term resolution of the problem, particularly if seed moisture content is ordinarily high at harvest.

Long Term

There is really no acceptable long-term alternative to solving the aflatoxin problem in Thai corn. The alternate crops such as grain sorghum being grown are of great value as feed supplements but have little value as exports. Although increased production of the second crop of corn harvested during the nonrainy season is an alternative, no data are available on the occurrence of aflatoxin in the second corn crop or in grain sorghum. Knowledge of the factors involved in aflatoxin contamination in Thai corn is essential for scientific and economic decisionmaking.

LONG-TERM SOLUTION VIA BIOTECHNOLOGY

Data from the United States, if verified, have demonstrated the presence of virus (double stranded-RNA) in a nontoxigenic strain of Aspergillus flavus (Schmidt et al., 1983). When treated with cycloheximide, this strain became a toxin producer. Attempts are now under way to transfer this ds-RNA to Aspergillus parasiticus to determine if it will prevent toxin production by formerly toxin-producing strains. If these results are promising and successful, then transfer of the ds-RNA to a corn cell via a t-plasmid or other method could create a source of resistance to aflatoxin formation in corn by Aspergillus flavus.

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ANNEX:

AFLATOXIN ANALYTICAL EQUIPMENT, GLASSWARE, CHEMICALS, AND SUPPLIES

Corn Grinder: Must grind it very fine.

Explosion-proof Waring Blender: Connected to an explosion-proof switch mounted 24 inches from blender. Can be mounted on a 24 x 30 inch board of 3/4 inch plywood or equivalent.

UV Viewing Cabinet: Chromato-Vu. Long- and short wave UV. (Dr. Thirayudh at the Department of Physiology, Mahidol University, has an excellent unit and preferable to the one of Dr. Dara.)

Drying Oven: Mechanical convection type for drying plates, etc.

Shaker for 500 ml Erlenmeyer Flasks: Special units available have 24/40 mm ground glass tops and polyethylene stoppers.

TLC Development Tanks: Need several, complete tops and racks. Latest types have multiplate racks.

Storage Cabinets: Special dessicating cabinets for plate storage.

TLC Spreading Board and Spreader Unit for 20 x 20 cm plates.

Glass Plates: Thickness varies. Purchased replacements locally from plate or double strength glass--20 x 20 cm polish edges for safe handling.

Separatory Funnels: 500 ml pyrex with polypropylene stopcocks and stoppers.

Sep-Pak Cartridge Filters for Cleanup of Extract.

Silica Gel GHR-Merck: Brinkman Instruments in the United States.

Aflatoxin Standards.

Solvent Chemicals: Acetone, chloroform, or methylene chloride, ethanol, methanol, toluene, formic acid varying with system for developing plates. Some needed only in small but very pure reagent grade about ACS quality.

3. UTILIZATION OF CASSAVA

INTRODUCTION

Cassava is a major crop in Thailand. Approximately 20 million tons of cassava were produced in 1984, mainly in the low-income areas of Northeast Thailand. Because exports to Europe were severely curtailed recently by the European Economic Community (EEC), alternative uses must be found for cassava and its products. Increased research and development activities in bioscience and biotechnology could be of considerable help in the utilization of this important crop.

OBJECTIVE

The objective of the research projects on cassava utilization outlined here is to identify areas of research and development that are very likely to provide improved and alternative uses of cassava. These projects could result in considerable advances by applying some of the newer techniques of bioscience, biotechnology, and related science and technology to transform cassava into value-added products for internal and export purposes.

GENERAL KNOWLEDGE

Approximately 92 percent of the cassava produced in Thailand is converted into chips and pellets, primarily for export, and the remaining amount is used for the manufacture of starch. Much of the research outlined includes new uses of the root and its flour, starch, and by-products (waste from starch manufacture, leaves, and stems). Some of the general knowledge of cassava flour, roots, stems, and leaves in Thailand is taken from the report of a seminar on a plan of cassava utilization for food and industrial products, held at the Thailand Institute of Scientific and Technological Research (TISTR), July 17, 1984.

Utilization of Cassava Flour

Manufacturing, Quality, and Specifications of Cassava Flour

Thailand is capable of constructing a cassava flour manufacturing factory that can produce the highest product quality. Thai construction firms have already constructed cassava flour manufacturing factories in Indonesia and China.

Thailand is supplying 80 percent of cassava flour to the world's market. In 1983, about 0.35 million tons were exported; 0.2 million tons were consumed locally. An estimated 92.7 percent of the cassava roots were transformed into pellets and chips and only 7.3 percent into cassava flour.

Since there are no problems with the processing technology, the production scale could be expanded if there is an increased demand for other uses. The standard and specifications of cassava flour could be adjusted upon request by the end users to meet their requirements. To reduce the cost of processing, good varieties of cassava with high starch content in the roots are desirable.

Utilization of Cassava Flour for Food

Because cassava flour has a low nutritive value in terms of protein, vitamins, etc., it cannot be used as a staple food in most countries. The exception, however, is several African countries where cassava flour is known as gari. TISTR was successful in producing gari but there were difficulties with its future exportation.

Small amounts of cassava flour are being used for mixing with other sources of starch flour to make noodles, breads, jellies, etc., but the poor chemical structure of cassava starch, which contains high amylopectin and affects the texture of the products, prevents the use of high proportions. Cassava flour could, however, be used in small quantities in food processing, e.g., for binding. The development of a new kind of snack food or instant food using cassava flour should be investigated.

Utilization of Cassava Flour in Industry

A number of factories are producing glucose and dextrin using the acid-enzyme and enzyme-enzyme processes and imported enzymes. These products are supplied to local factories, especially confectioneries. The cassava flour used must be selected from certain factories according to its sulfite content. A high content of sulfite will prolong the time for saccharification because of low enzyme activities.

A large amount of cassava flour is used in the paper, fiber, and textile industries for sizing purposes. However, such applications face problems with water pollution, especially in the recycling process.

A certain amount of cassava flour is used for the manufacture of adhesives having applications that compete with those of synthetic

adhesive materials. Thus, further investigation of this use of cassava flour is recommended.

In summary, the uses of cassava flour are limited, especially for the food industries. Expanded utilization of cassava flour will likely not occur in the near future unless new industrial products can be developed using cassava flour as the raw material.

Utilization of Cassava Roots

Large portions of the cassava crop are used for animal feed, but this product faces stiff competition from other sources of carbohydrates which have a higher nutritive value. Cassava is also often excluded from the main rations because of its low protein content and vitamins compared with other sources. Fermentation of cassava by microorganisms to increase its nutritive value has been proposed. The protein content of the product could be raised by 15-20 percent, enhancing its use as the major feed ration.

Production of crude alcohol for exportation is another possible use of cassava roots. However, more research is needed to reduce the cost of production and to solve pollution problems. Production of alcoholic beverages may be more difficult from the viewpoints of quality and marketing. Yet, another possibility is the use of alcohol as an octane enhancer in place of lead compound to alleviate environmental pollution.

Citric acid fermentation faces the problem of increased cost because residues from cassava flour factories are used as the raw material. Research on citric acid fermentation from cassava root by solid culture or submerged culture is needed to solve this problem.

Production of lysine from cassava will be undertaken by one factory next year, requiring about 800,000 tons of cassava root per year. More research should be done along this line on fermentation of other amino acids or on other types of product diversification.

Utilization of Cassava Stems

The Department of Forestry has conducted research on the use of cassava stems in paper pulp and fiberboard manufacture. These processes are highly feasible for implementation at the small-scale level in rural areas as has been done in the People's Republic of China, and research should be continued on the development of small-scale technologies for the local manufacture of fiberboard from cassava stems. At the same time, harvesting and packaging processes must be developed to reduce the cost of transportation.

Utilization of cassava stems for energy sources, such as charcoal, and developing gasification process, etc., are also important areas for further investigation.

Utilization of Cassava Leaves

Cassava leaves have a high protein content, over 20 percent, but they also contain a large amount of hydrocyanic acid which is toxic to animals. They can be used as animal feed after microbial fermentation, which reduces the cyanide content. Special treatment of leaves as well as formulations of leaf rations in feed need further investigation. Harvesting and packaging tools should also be developed for this purpose. The fact that harvesting of all parts of the crop depletes soil minerals should be considered carefully since it has been suggested that cassava leaves and residues could fulfill partial requirements for the fertilizers needed for plantations.

In summary, planning for the utilization of cassava will benefit from research on and the development of new products as well as ways to increase the ration of cassava in existing processes. Modes of utilization should be directed toward the local supply of industrial feedstocks and exportation of the diversified products.

CAPABILITY

The presently existing National Cassava Policy Committee is concerned with all aspects of cassava utilization in Thailand. This committee is served by the Cassava Utilization Research Subcommittee for which TISTR serves as the center and coordinator with various governmental agencies and universities (including Kasetsart, Khon Kaen, Mahidol, and Chulalongkorn). Research on cassava is now being carried on at many locations in Thailand. However, most of the cassava utilization pilot studies are being carried out at TISTR.

CONSTRAINTS

- Insufficient research on bioscience and biotechnology applied to cassava
- Insufficient developmental equipment and facilities for scale-up of promising products
- Inadequate translation of discoveries from universities and institutes to industry
- Shortage of well-trained scientists in some disciplines, e.g. genetic engineering
- Inadequate marketing and economic information
- Lack of specialists in certain areas including technology transfer

PROJECTS

PROJECT: Modification of Cassava Starch to Take on the Chemical and Physical Properties of Competing Starches such as Imported Potato Starch

About 10,000 tons of modified potato starch are imported to Thailand every year for use in food factories to prepare many products including transparent noodles. This imported product could be replaced by successfully modified cassava starch. Slight variations can be introduced into unmodified starch by adjusting pH, by mild heat treatment, or by adding small quantities of chemicals or adjuvants before or after drying. This starch will then perform more effectively in specific applications. For example, common starch intended for enzyme conversion may be adjusted to a specific pH range and small amounts of inorganic salts that facilitate enzyme action may also be added. Starches used in food use are also often pH-adjusted.

Unmodified cooked starch has such great thickening power that pastes containing more than 4-5 percent solids are too thick to handle. Further, such pastes gel very rapidly when cooled. For many uses, pastes containing higher solids with a reduced tendency to thicken or with the ability to form softer gels are required.

One method used commercially to reduce the viscosity of starch pastes is the acid modification process developed by Duryea and patented in 1899. For this method, the starch, suspended in water and agitated, is subjected to mild treatment with dilute mineral acid at elevated temperatures (below the gelatinization temperature) for varying periods of time. When tests show that the desired viscosity has been reached, the acid is neutralized with sodium carbonate and the starch is filtered, washed, and dried. In this manner, a series of starches yielding pastes of decreasing viscosity are obtained.

Another method for reducing the viscosity and altering the properties of starch is oxidation. Several oxidizing agents such as chlorine, hydrogen peroxide, and potassium permanganate have been investigated.

Frequently, oxidized starches are made using sodium hypochlorite as the oxidizing agent. As in the case of acid modification, aqueous starch suspensions under continuous agitation are treated with dilute sodium hypochlorite containing a small excess of caustic soda. The reagent solution is added slowly to the starch suspension in a reactor which is maintained at about 120°F. Cooling coils in the reactors remove the heat produced by the oxidation. When the correct amount of reagent has been added and sufficient time for reaction has elapsed, the viscosity of the starch is determined. When the desired degree of oxidation is reached, the starch slurry is treated with sodium bisulfite, adjusted to the desired pH, filtered, washed, and dried. Products with a wide range of modification are produced.

Oxidized starch retains its original granule structure and is still insoluble in cold water. It is extremely white due to the bleaching action of the sodium hypochlorite. In addition to having decreased viscosity, oxidized starch pastes are relatively clear and show a reduced tendency to thicken when cooled. When dried, oxidized starch films are clear and tough; they are sometimes referred to as gums.

Treatment of starch with sodium hypochlorite brings about a random oxidation of a limited number of hydroxyl groups to carboxyl or carbonyl groups, with the resulting rupture of the adjacent glucosidic bond. Since the oxidation occurs in the presence of excess sodium hydroxide, the carboxyl groups are neutralized, resulting in a sodium salt. Because the sodium salt of the carboxyl group is bulkier than the parent hydroxyl group, it is postulated that the tendency of the amylose molecules to associate and retrograde into gels is reduced.

The major uses for oxidized starches are in the paper industry, in the textile industry, and as components of adhesives. In addition, some types find use in food applications where high solids and low viscosity are desired.

Human resources required: Two starch chemists, one food technologist, one process engineer, and one technology transfer specialist.

Duration: Short term*

PROJECT: Feasible Process for the Production of Pharmaceutical-Grade Dextrose (Anhydrous)

Because anhydrous dextrose is not manufactured in Thailand, nearly 500 tons of this product are imported for medical uses. Anhydrous dextrose is usually produced by redissolving dextrose hydrate and refining the resulting dextrose solution. This solution is evaporated to a very high solids content and anhydrous a-D-glucose is crystallized on induced or added seed crystals at elevated temperatures. The anhydrous dextrose is separated by centrifugation, washed, and dried.

Human resources required: One biochemist, one chemist, two process engineers, and one technology transfer specialist.

Duration: Short term*

PROJECT: Development of Xanthan Gum from Cassava for Use in Food and Petroleum Recovery

Thailand imports xanthan gum; however, bioresources such as cassava are in abundant supply in Thailand and could be used as a raw material for this product.

Xanthan gum is a polysaccharide produced by culturing X. campestris NRRL B-1459 on a well-aerated medium containing commercial glucose, organic nitrogen sources, dipotassium hydrogen phosphate, and appropriate trace elements. When fermentation is finished, the culture fluids have a viscosity as high as 7,000 centipoises, and they appear homogeneous and have very short flow characteristics.

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*Short term is one to three years; long term, over five years.

This macromolecular polysaccharide is composed of D-mannose, D-glucose, D-glucuronic acid (as the potassium salt), and a small proportion of acetyl groups. It can be produced on an industrial scale and is stable in storage. Analytical fractionation indicates fairly sharp molecular distribution for the native polysaccharide. The polysaccharide forms homogeneous dispersions in water which show plastic rheological properties and viscosity comparable with that of high-grade plant gums. Outstanding characteristics of practical significance are the atypical insensitivity of solution viscosity to salt effects and to heat, especially when salt is present. Solutions of low concentration show a restricted viscosity decrease upon the addition of salt; those of higher concentrations show substantial increases. Viscosity is enhanced still further by monovalent cations at basic pH and by divalent cations at neutral or slightly basic pH. Salt moderates or eliminates any decrease in viscosity due to heat and, in somewhat higher concentrations, it increases the viscosity of heated solutions. Heating or deacetylating polysaccharide B-1459 causes no impairment of its properties but, rather, actual improvement. Glucose from cassava starch could be used in the fermentation.

Human resources required: One biochemist, one chemist, one microbiologist, one food technologist, one process engineer, and one technology transfer specialist.

Duration: Short term

PROJECT: Improvement of Snack Items and Breakfast Cereals to Include Cassava Starch

Snack items and breakfast cereals are popular food products, but many of them are imported. Cassava and its starch could be used in the preparation of these food products, including the utilization of hot and cold extrusion techniques, blended and enriched with other food ingredients. Moreover, for these purposes, other local ingredients such as rice, bean, and corn are readily available. These products can be enriched with vitamins and minerals for better nutrition.

Human resources required: Four food scientists and technologists, one chemist, three process engineers, and one technology transfer technologist.

Duration: Short term

PROJECT: Bioconversion of Cassava, Including Starch, Leaves, Stems, and By-products, by Ordinary and Genetically Improved Microorganisms to Produce Food, Feed, and Industrial Products

With the advent of bioscience and biotechnology, it should be possible to greatly increase the utilization of cassava and its products. Improvements in a cassava product or process could result in production

of the following materials: animal feed, feed supplements, food, alcohol, organic acids (citric), food colors, enzymes (cellulose, pectinase, xanthanase), amino acids (lysine, glutamic acid), biological pesticides, plant growth substances, and antibiotics.

The cassava plant has commanded considerable attention in Brazil as a source of starch for fermentation. Cassava roots contains 20-35 percent starch and 1-2 percent protein. The average crop production in Brazil is 13 tons of roots per hectare. The feasibility of alcohol production from starch materials to compete with Brazil's already successful sugarcane process will depend principally upon the optimization of the liquefaction and saccharification steps in the manufacturing process.

Starch hydrolysis is a problem in the cassava process, even though the cassava starch is readily susceptible to alpha-amylase. The cassava root fibers create a barrier to starch hydrolysis when whole roots are used for fermentation, but they can be removed via biological pretreatment with the cellulolytic microorganism Trichoderma viride. A fermentation broth of Basidiomycete and T. viride has increased both the rate of sugar formation and degree of solubilization, with subsequent a decrease in substrate viscosity.

About 400 million lb/year (180 million kg/year) of citric acid are produced in the United States and an additional 150 million lb/year (68 million kg/year) in the rest of the world. Commercially, about 90 percent of citric acid is produced by either submerged or surface fermentation. Most new fermentation plants use submerged fermentation. In surface fermentation, spores of the fungus Aspergillus niger are inoculated on the surface of an appropriate medium in large pans that are about 2-3 inches deep. After inoculation, the shallow pans are incubated still at 25-30°C for 7-10 days, after which the fermentation is complete.

The yields of citric acid, based on the amount of sugar consumed, depend on the substrate. Yields from submerged and surface fermentation of various sugars by A. niger are for the fermentation step only and range from about 70 percent in four days to 95 percent in 6-14 days. After the surface culture fermentation is complete, the liquor is decanted, the mycelial mat is washed, and the wash liquid is added to the fermentation solution.

The only major difference between submerged and surface fermentations is that air must be supplied to the submerged culture at a rate of 0.5-1.5 VVm. The pH from both types of fermentation drops from 4 to 2 or below during a fermentation period of 5-14 days. The media are the same for each type of fermentation. The sugar to be fermented is present in concentrations of 15-25 percent w/v. Nitrogen is incorporated into the medium as an inorganic salt, or it is supplied in the substrate in an organic form with concentrations no higher than 0.08-0.09 percent w/v. Phosphate, iron, manganese, magnesium, and zinc must be carefully controlled in the medium to assure good citric acid yields. Magnesium is usually supplied as $MgSO_4 7H_2O$ at about 0.1 percent and potassium as KH_2PO_4 at 0.05-0.2 percent. Iron concentrations should not exceed 2-5 mg per liter of medium.

Other fermentatone methods and microorganisms are reportedly efficient in citric acid production. The two products ethanol and citric acid were used above only to illustrate the research activities anticipated under this project.

Human resources required: Microbiologists, biochemists, chemists, process engineers, and technology transfer specialists. (The number of scientists needed will depend upon products to be investigated.)

Duration: Long term

PROJECT: Bioregulation of Cassava for Improved Tuber Quality (Low Cyanide, Higher Starch, Lower Fiber, and Higher Protein Content Using Tissue Culture and Plant Growth Regulator Techniques

Decreased cyanide and fiber contents and increased starch and protein contents will improve the quality of cassava. These improvements are possible with the use of plant regulation, an emerging technology which has advanced rapidly in recent years. Plant regulators are being applied commercially in weed control and as yield and quality enhancers. They have proved very beneficial in horticulture and are being developed for agronomic crops.

Plant regulators are organic compounds, other than nutrients, which promote, inhibit, or otherwise modify any physiological process in the plant. They control growth or alter physiological activities to make plants more efficient in the use of water or nutrients or better able to withstand environmental stresses. As used in agricultural production, plant regulators may be biostimulants, retardants, yield enhancers, harvest aids, and quality improvers. They may influence genetic expression, propagation, growth, reproduction, yield quality and quantity, harvest, and storage.

To advance the role of plant regulators in cassava technology, it is essential to understand their modes of action in physiological processes and to make practical application of this knowledge. Promotion of plant regulators to a leading role in cassava production requires intensive research conducted along four lines:

1. Identify the physiological processes influenced by plant regulators and synthesize potential regulants for testing.
2. Give special attention to yield enhancers and their modes of action.
3 Study the ability of some plant regulators to ameliorate environmental and biological stresses.
4. Analyze practical applications in relation to other cultural practices and to conservation and economic impacts.

Human resources required: Three plant physiologists, three biochemists, one agronomist, and a plant growth regulator specialist

Duration: Long term

PROJECT: Production of Chemical Derivatives of Cassava Starch to Give Unique New Products and Properties Suitable for Expanded Applications in Encapsulation of Pesticides, Paper Additives, Textile Sizes, Binders, Extenders, Water Absorption, etc.

New and expanded markets for cassava starch could be realized by preparing new, unique derivatives for meeting some of the market needs. Since the starch molecule contains many primary and secondary hydroxyl groups, it can be modified by chemical derivatization. Unlike the modifications thus far discussed, derivatization may or may not reduce the viscosity of the parent starch. Derivatization is used to impart properties to the derivative different than those of the parent starch, thereby allowing the derivative to meet the requirements of specific end uses more effectively. Countless starch derivatives have been described in technical literature and in patents, but only a limited number are manufactured and used commercially.

The derivatization of starch differs from most chemical modifications of polymers in that the changes in properties are attained with very slight changes in the molecule itself. In fact, all commercial derivatives are prepared under such mild conditions (usually in aqueous suspensions) that the starch granules retain their integrity. This allows the products to be handled in processing and application in much the same manner as the common starches previously discussed.

Starch derivatives are almost always prepared by adding the desired reagent to an agitated suspension of starch in water. By adjusting the pH of the slurry with an alkali, and sometimes with a catalyst, the mild reactions proceed on the ungelatinized starch at only slightly elevated temperatures. After sufficient reaction time, the derivatives are recovered by filtration or centrifugation, washed with water, dried, and packaged.

Two basic types of derivatives are prepared commercially: cross-linked derivatives and stabilization derivatives. Cross-linked starches are made to overcome the sensitivity of starch sols to shear and processing conditions. This is accomplished by treating starch in the granule state with trace amounts of difunctional agents capable of reacting with hydroxyl groups on two different molecules within the granule. Reagents such as phosphorus oxychloride or sodium trimetaphosphate may be used as cross-linking agents. Very small amounts of these agents can exert a marked effect on the behavior of the cooked starch. The degree of cross-linking controls the rate and extent to which starch swells in cooking. Cross-linking decreases the sensitivity of starch sols to temperature agitation and acids, improving resistance to loss in viscosity.

Starch is stabilized against gelling by using monofunctional reagents which react with hydroxyl groups on the starch to introduce substituent groups which interfere with intermolecular association between starch molecules. Certain reagents may also introduce specific functionality into starches, e.g., increasing their water-combining capacity or viscosity or imparting a positive charge to the starch molecule.

The following are examples of starch derivatives:

Hydroxyethyl Starches. To produce hydroxyethyl starch, a starch slurry is adjusted to an alkaline pH and a salt is added to suppress the tendency of the starch to gelatinize. Ethylene oxide in varying quantities is added slowly to the agitated slurry and allowed to react for the proper time. Most hydroxyethyl starches are also acid-modified to reduce their viscosity. The hydroxyethylated starch is recovered by filtration, washed, and dried. The introduction of the hydroxyethyl group reduces the gelatinization temperature of the starch and results in clear, stable pastes which are used widely in surface sizing and coating paper.

Cationic Starches. Reaction of starch with tertiary or quaternary amines yields quaternary ammonium or amino alkyl starches. When dispersed, these starches yield positively charged particles that are strongly adsorbed by negatively charged cellulose fibers in the manufacture of paper. Less starch is used, but, more important, nearly all of the cationic starch in solution is adsorbed by the paper, leaving very little in the effluent going to the waste disposal system. In addition, cationic starch promotes the retention of fillers and pigments in the sheet while reducing the loss of very fine paper fibers. The additional retained fiber and the ability of the starch to bond the cellulose fibers together give greatly increased internal strength to the sheet. This substantive characteristic of cationic starches also makes them useful as surface sizes and as the adhesive in pigmented coatings.

Starch Acetates. Starch can be acetylated with acetic anhydride or vinyl acetate under carefully controlled conditions of pH, temperature, and time. After reaction, the starch is isolated by filtration, washed, and dried. Sufficient acetyl groups are introduced to prevent retrogradation of the starch paste. Acetylated starches are used to size textile warps, yielding tough, flexible yarns. The reduced tendency to congeal makes starch acetates easy to pump and to apply at the slasher.

Starch acetates are also used as food starches. For example, waxy maize starch can be cross-linked with phosphorous oxychloride and then acetylated with acetic anhydride or vinyl acetate to produce an excellent thickener, texturizer, or stabilizer used in preparing a wide variety of products.

Starch Succinates. The use of succinic anhydride instead of acetic anhydride yields starch succinates which are also used as thickening agents for foods. The 1-octenyl succinic ester produced has an affinity for fats and oils superior to that of other derivatives.

Starch Phosphates. Starch can be esterified with monosodium orthophosphate or sodium tripolyphosphate to yield starch phosphates that produce gels more stable than those produced from the parent starch. The phosphated starches are used mainly in preparing food products.

A wide variety of monomers have been graft polymerized onto granular and gelatinized starch, and several of the grant polymers show promise as thickeners for aqueous systems, flocculants, clarification aids for wastewaters, retention aids in papermaking, and many other uses. The polymer that has received the most attention and is now being marketed by several U.S. companies is made by graft polymerizing acrylonitrile onto gelatinized starch and subjecting the resulting starch-graft-polyacrylo-nitrile copolymer to alkaline saponification to convert the nitrile groups to a mixture of carbamoyl and alkali metal carboxylate groups. Removing the water from this polymer provides a solid which absorbs many hundreds times its weight in water but which does not dissolve. Because of its ability to absorb such large amounts of water rapidly, this polymer has been named "Super Slurper." The U.S. Department of Agriculture has granted more than 50 nonexclusive licenses to parties interested in practicing the technology covered in the four patents issued in 1976 on work with this starch graft polymer.

Agricultural applications such as seed and root coating and as an additive to fast-draining soils to retain water appear most promising for Super Slurper. Large-scale field trials with corn, soybean, and cottonseed coated with the polymer have shown increased germination and seedling emergence, and in most trials increased yield. The dipping of bare-rooted seedlings in hydrated polymer before transplanting overcomes transplant shock and greatly increases survival.

Human resources required: Four chemists, two process engineers, and technology transfer specialists

Duration: Long term

PROJECT: Improving Laboratory and Production Processes of Glucoamylase, Glucose Isomerase, and Alpha-Amylase from Ordinary and Genetically Improved Microorganisms for the Conversion of Cassava Starch to Glucose or Other Fermentation Sugars

These enzymes are currently imported with a likelihood of increased usage. The major use of starch for enzyme conversion is found in manufacturing plants where each year billions of pounds of starch are converted to nutritive carbohydrate sweeteners. These processes utilize alpha-amylase, beta-amylase, glucoamylase, debranching enzymes, and isomerases. The manufacture of sweeteners from starch is a multistep process which begins with the conversion of a starch slurry to a low solids content syrup or liquor. The starch conversion process is halted when the desired saccharide composition is reached.

The most common methods employed in the commercial production of glucose syrups are the acid process, the acid-enzyme process, and the multiple enzyme process. In the acid-conversion process, a starch slurry of appropriate dry substance is acidified to attain a pH of about 2 and is then pumped to the converter. The process is terminated by reducing the pressure and neutralizing the resulting liquor. Small amounts of sodium chloride are produced by this neutralization. The

liquor is then clarified to remove suspended solids and is concentrated by evaporation to an intermediate density. The intermediate syrup is further clarified, decolorized, and finally concentrated in evaporators to finished density. Some syrups are treated with ion-exchange resins for further refinement.

The acid-enzyme process is similar except that the starch slurry is only partially converted by acid to a given D.E. (dextrose equivalent). The "light liquor" is then treated with an appropriate enzyme or combination of enzymes to complete the conversion. For example, in the production of 42 D.E., high-maltose syrup, the acid conversion is halted at a point where dextrose production is negligible. Then the maltose-producing enzyme (beta-amylase) is added and the conversion continued under appropriate conditions to the desired level. The enzyme is then deactivated and the purification, clarification, and concentration procedures are continued as in acid-converted syrup production.

In multiple enzyme processes, starch granules are gelatinized and the preliminary starch splitting or depolymerization is brought about by an alpha-amylase enzyme rather than by means of acid.

Various intermediate syrups of differing composition may be further converted with enzymes having specific modes of action to provide particular types of end-products such as high-maltose syrups, high fermentable syrups, and others.

In the manufacture of high-fructose corn syrup, dextrose solutions or high D.E. substrates produced by acid-enzyme or dual enzyme processes are further treated enzymatically with a suitably purified isomerase before concentration. Isomerization is usually carried to a point where nearly half of the dextrose in the substrate is converted to fructose. Following this step, the product is finished as previously described with the addition of an ion-exchange refining step. Thus, a high-fructose corn syrup with a sweetness roughly comparable to sucrose is produced.

Human resources required: Two biochemists, two microbiologists, one process engineer, and one molecular biologist

Duration: Long term

PROJECT: Liquid Waste Disposal of Cassava By-products by Such Techniques as Membrane Separations and Microbial Conversions to Single-Cell Proteins Using Ordinary and Genetically Modified Microorganisms to Give Animal Feed and Feed Supplements

Waste disposal of cassava by-products, both liquid and solid, are currently very serious problems. The solid waste is more easily converted to animal feed. The liquid waste needs considerable additional research to aid in the manufacture of cassava products and to greatly decrease pollution.

Ethanol production from plant biomass is being studied extensively by various research laboratories throughout the world. Some researchers are pursuing the production of both single-cell proteins and alcohol from agricultural wastes by utilizing various biological conversion processes. This research also includes thermophilic bacteria that produce ethanol from xylose, mixed culture fermentation, and the use of Clostridium thermocellum.

Human resources required: Microbiologists, biochemists, chemists, sanitary engineer, process engineer, and technology transfer specialist (The exact numbers depend on the size of the effort.)

Duration: Short term

4. DEVELOPMENT OF ANIMAL BIOTECHNOLOGY IN THAILAND

INTRODUCTION

Thailand currently imports 95 percent of its milk products, mainly in the form of powdered milk, making it highly desirable to increase fluid milk production and lower imports. The current average milk production per cow in Thailand is approximately 2,400 kg over a 305-day lactation period. This compares to milk production of 7,560 kg for cows in the U.S. state of California and 7,640 kg in the state of Washington.

Approximately 20 dairies are located in and around the major metropolitan area of Bangkok, supplemented by hundreds of rural farms having one or two cows. The number and type of livestock and the rate of artificial insemination are given in Table 2.

TABLE 2 Number and Percentage of Animals Having Artificial Insemination (AI) in 1983.

Animal Type	Total Number (000)	Number of Inseminations	Covering Rate of AI (Percent)
Dairy cattle	60,000	41,291	45.5
Beef cattle	5,062[a]	20,555	0.82
Buffalo	6,299[a]	2,247	0.08
Swine	5,386[a]	9,990	0.31

[a]In thousands.

As shown in Table 2, less than one-half of the dairy and 190 of beef cows are artificially inseminated. Although artificial insemination has been practiced for 30 years in Thailand, conception rates and the genetic quality of semen are low. This situation, coupled with the nutritional and management problems of a dairy industry in a tropical environment, accounts for the relatively low level of milk production in Thailand.

STATE OF THE ART OF ANIMAL BIOTECHNOLOGY IN THE UNITED STATES

Recent advances in gene transfer, molecular genetics, and recombinant DNA technology make it possible to alter genetic potential and the expression of this genetic potential in domestic animals. This breakthrough promises to revolutionize animal agriculture and contribute to more efficient and profitable production and higher quality animal products. The use of genetic engineering as a tool to accomplish these goals is receiving increased emphasis in both industry and federal and state-supported research programs. To take full advantage of genetic engineering technology to enhance animal production, basic information is needed in the areas of genetics, reproduction, animal growth, and nutrition.

During recent years, research has clearly demonstrated the potential role of germ cell and embryo manipulation and transfer for livestock improvement. The exchange or replacement of genetic material in gametes or early embryos holds promise of greatly increasing the rate of genetic improvement and making genetic combinations beyond those possible through natural fertilization processes. The benefits of freezing embryos for storage and transport will be greatly enhanced if embryos can be divided into identical twins, triplets, etc. and their sex predetermined. Currently, this technology is being applied in a rapidly growing embryo transfer industry. Approximately 10,000 bovine pregnancies were produced in North America in 1978 by embryo transfer and approximately 18,000, or 80 percent more, in 1979 with a commercial value of about $20 million.

Additional basic information on the morphology and physiology of germ cells, embryos, and their maternal environment, and on their modification, is needed for more efficient production of meat, milk, and wool. Regional coordination of such research is vital as well to achieve optimum output and minimum duplication. Communications among researchers with common goals will lead to objective evaluation of proposed research and will avoid costly errors.

PREVIOUS WORK ON GAMETE DEVELOPMENT AND IMPROVED EMBRYO PRODUCTION

Embryo production, sexing, storage, multiplication, and transfer technology could accelerate genetic improvement of livestock by providing large numbers of genetically superior offspring from individual females. What follows is a brief review of the current status of research on the objectives of the project proposed in this working group report: gamete development and improved embryo production.

Recovery of large numbers of ova from ovaries of livestock species requires that the recovered ova undergo maturation, fertilization, and development in the laboratory to a stage where the embryos can be sorted or transferred. Present technology allows only slight success in completing each of these three steps. However, the three have not been applied together in domestic animals, and much improvement is needed in each category.

Maturation of ova is arrested from early fetal life until a follicle containing a specific ovum is selected for ovulation. Ova will resume nuclear meiosis and undergo maturation upon their removal from the ovarian follicle or within follicles; that is, when inhibiting substances are removed by natural or artificial administration of luteinizing hormone to the follicle. Nuclear maturation alone is not sufficient for fertile ova. It must be accompanied by maturation of the cytoplasm and loosening of follicle cells around the ovum. This change in the loosening of follicular cells is induced by follicle-stimulating hormone.

While some ova matured outside the body have been fertile, considerable work is needed to determine how maturation is controlled if a real breakthrough is to be made in the harvest and use of thousands of ova from a single female (Midgley and Sadler, 1979; Pond et al., 1980).

Ova harvested in large numbers and matured *in vitro* would be most efficiently utilized by fertilization *in vitro*. Elimination of the surgical procedures involved with fertilization of these ova *in vivo* as well as the basic understanding of developmental processes make *in vitro* fertilization the method of choice.

Techniques for fertilization outside the body have been developed for the hamster, mouse, rat, rabbit, guinea pig, dog, cat, human, and nonhuman primates, and recently the cow. These techniques operate with various efficiencies and stages of success in each species. Live offspring have resulted from such fertilization only in the mouse, rat, rabbit, and human, and in one case cattle and sheep. In domestic animals, various pieces of the needed technology have been developed, but overall success rates remain very low.

Much research is needed on improving fertilization rates in the laboratory. Most experiments on cattle failed to determine if the fertilized ova would develop into live offspring. The rate of fertilization was less than perfect, and reactions in the ovum were not entirely normal. Research is needed to (1) perfect the technology for fertilization of cow ova, including study of normal physiological events such as capacitation of sperm, acrosome reaction of sperm, and cortical granule reaction in the fertilized egg; (2) develop fertilization methods for sheep and swine; and (3) develop technology to produce normal young from ova fertilized outside the body.

The tools needed to perfect methods for fertilization outside the body and to develop an understanding of the fertilization process are now available (e.g., the ability to accomplish fertilization of one egg by one sperm, and the ability to separate and identify several components of the fertilization process). Besides the obvious application of increasing numbers of fertilized ova available for embryo transfer, fertilization under laboratory conditions can be useful in studying such things as variation in fertility among sperm (Pond, Merkel, McGilliard, and Rhodes, 1980; Wright and Bondioli, 1981).

The present culture systems used for *in vitro* fertilized ova and embryo of later cell stages are suboptimal for development in all livestock species as well as in the human. This deficiency accounts in large part for the low *in vitro* fertilization and conception rates following culture (Wright and Bondioli, 1981; Bondioli and Wright, 1983).

In addition, culture systems that can support development are essential to the success of embryo manipulation procedures to determine embryo viability.

Livestock Improvement by Embryo Manipulation

There is some evidence that chimeras--animals produced by mixing cells from two or more embryos--are produced in livestock species by natural events. For example, fusion of fetal blood vessels can occur with multiple births in cattle with the migration of blood-borne elements. It is possible that some blood-borne elements could be primordial germ cells, and with twin bull calves such an interchange of germ cells could develop to spermatozoa resulting in one bull siring a number of his brothers' offspring (Rowson, 1973).

Advantage can also be taken of the natural production of chimeras in most instances of bovine twin pregnancies, even when the twins concerned are both heifer calves. Such calves, which can be of markedly differing breeds (Jerseys, Friesians), would show tissue tolerance to each other because of the chimerism. It is possible to investigate questions bearing on various milk production characteristics, not only by examining what happens as a result of erythrocyte chimerism but also by looking at the reciprocal interchange of half the udder (by grafting operations), thus shedding light on milk production at the cellular level (Rowson et al., 1971).

Apart from the chimeras produced naturally in instances of multiple pregnancies in cows, there is the possibility of artificially producing such animals. Attempts have been made in various mammalian species in recent years to produce chimeras by the injection of embryonic, fetal, and adult cells into developing eggs and blastocysts. The feasibility of this technique, which could be a valuable research tool, has been well demonstrated in mice (Gardner and Munro, 1974a), rabbits (Gardner and Munro, 1974b), and sheep (Tucker et al., 1974).

The technique of injecting such cells is relatively simple, and the blastocyst at this stage is quite incapable of an immune response. It is possible perhaps in cattle that adult cells from a particularly valuable cow could be injected into embryo to establish tissue tolerance between the cell donor and the calves that are eventually born. This might permit the subsequent successful grafting of ovarian tissue from the cell donor into the sterilized ovaries of the chimeric cattle, thus opening the way to proliferation of a particularly valuable strain by grafting operations.

Embryo transfer could be employed to produce identical twins, triplets, or possible litters of even greater size, not necessarily with one recipient having to carry all the identicals. Such production could follow the separation of individual blastomeres at, say, the four-cell stage, and transfer of these individually into suitable recipients. Several studies along these lines have already been reported for the rabbit and the pig (Moore et al., 1968.) It is in cattle, however, that the greatest interest in the production of identicals rests. For many years, bovine monozygotic twins have been used in several areas of

research in animal production. Among the various problems in getting isolated blastomeres to develop is that of providing them with a suitably sealed zona pellucida (using the rabbit oviduct location to provide a mucin plug has been attempted) if they are to survive transfer at an early cleavage stage or of developing them in the laboratory to the blastocyst stage, at which time they may survive transfer in the absence of the zona pellucida.

The availability of more information in the years ahead is likely to bring many exciting developments in ovum physiology. Nuclear transplantation is one technique which could have an immense impact on cattle breeding by permitting the precise and immediate selection of a particularly valuable genotype. Although the technique of nuclear transplantation was initially developed in studies with the large amphibian egg (King, 1966), recent work reported by Bromhall has shown some limited success in the introduction of the nuclei of embryonic rabbit cells into unfertilized rabbit eggs by both microinjection and virus-induced fusion (Bromhall, 1975). Although the difficulties in working with the minute mammalian egg are formidable, it is perhaps not impossible to contemplate a future in which calves can be obtained from eggs provided with transplanted nuclei. Not only would this give the required desirable genotype, but it would also simultaneously permit sex control, this being decided according to whether nuclei are taken from somatic cells of the male or female bovine. All this could have rather interesting implications for breeding and animal production generally.

Dissemination of Disease-Free Embryos

Until 1972, when mouse embryos were shown to survive storage at -196°C, the few attempts to preserve mammalian embryos at low temperatures had met with limited success. These earlier investigators indicated that short-term storage at temperatures above freezing point was more successful than storage at lower temperatures, but observations were made of small numbers of embryos and no assessment was made of the most suitable preimplantation stage(s) for preservation.

In cattle, sensitivity to cooling and storage appears to decrease during preattachment development. Survival at the blastocyst stage is high, with 92 percent, 67 percent, and 48 percent surviving storage for 30 minutes, 24 hours, and 48 hours, respectively (Trouson et al., 1976a). Reports on the survival of morulae are variable and may reflect differences in the actual developmental stage of the embryos at the time of collection. Only limited development of earlier cleavage stages was observed, but results indicated that slow cooling increased the survival rate although the differences were not significant (Moore et al., 1969).

In sheep, only one report thus far indicates that early embryos (2- to 16-cell) are more sensitive to cooling to 0°C than morulae stages, but the number of embryos used in the study was small. Other studies have shown that early sheep embryos, especially the 8- to 16-cell stage, store quite successfully at temperatures ranging between 0°C to 13°C for periods up to 20 days. Varying the cooling rate to 5°C does not appear to affect subsequent survival of sheep embryos (Moore and Bilton, 1973).

In goats, late morulae and early blastocysts stored at 5°C for one to two days, continued development in vitro and kids were born following transfer. Cooling of earlier stages was not examined (Bilton and Moore, 1976).

The major constraints and benefits to be gained from freezing reproductive cells are discussed in a later section of this paper (Storage of Eggs, Sperm, and Embryos, page 61). Early embryonic death, known to be a cause of infertility for many years, was addressed by Casida and coworkers in a landmark publication that appeared in the early 1950s (Casida et al., 1950). The importance of infectious agents to this problem was recognized when Camphylobacter fites was described as a cause of infertility and early embryonic death. Since that time, several agents, ranging from protozoa to viruses, have been identified as the cause of early embryonic death. Little is known, however, of the effects of viruses on embryos and on the earlier stage of the conception. There is increasing interest in this research due to the development of electron microscopy, metabolic procedures, and immunological techniques, but publications are few (Bowen, 1979; Evermann et al., 1981).

OBJECTIVES TO IMPROVE THAILAND'S CAPABILITIES IN BIOTECHNOLOGY

Immediate

1. To introduce Holstein embryos into Thailand from the United States to demonstrate the biotechnology-embryo transfer process using Thai researchers and private industry.

2. To offer immediate training in embryo transfer (six months) to one or more Thai researchers at Washington State University or other appropriate locations.

3. To establish several biotechnology research efforts aimed at improving overall reproductive efficiency in Thailand with marketable application to other tropical agricultural systems.

Long Range

1. To establish a core of scientists from existing Thai faculties and a scientific exchange program between Thailand and the United States, including graduate student training, and to study and perform animal biotechnology research.

2. To develop a type of dairy and beef animal that will yield maximum milk and meat production under Thailand's agricultural conditions. These engineered animals would most assuredly be in demand by other countries in tropical environments.

3. Accomplishing objectives 1 and 2 would provide the linkage between research and industry, resulting in increased milk and meat production and the exportation of superior genetic livestock.

Plans for Accomplishing Immediate Objectives

Objective 1: Several researchers at Kasetsart University (Drs. Vanda Sujarit and colleagues) and Drs. Prasert Songsasen and Samphah Singhajan have been trained or have had limited experience in embryo transfer technology. Although their several attempts at transfer with frozen and fresh embryos have met with no success, their understanding of the basic methodology is adequate. A private dairy located near the Kasetsart University Research and Development Institute (KURDI) and owned by Mr. Udom Vangtal seems to be typical of those found in Thailand. Although the attempts of this dairy at embryo transfer have not met with success, it does have some experience in the necessary methodology.

Embryos sent from the United States could be transferred within the next three months. Twenty embryos from superior Holstein females (purchase price of $1,250 each) could be transferred into native Thailand recipients, resulting in 8-10 pregnancies. In addition, a demonstration using embryos collected from two or three of Mr. Udom Vangtal's best cows could be transferred during the sire visit. With proper coordination, scientists from Kasetsart University, KURDI, and agricultural artificial insemination centers could all be involved in a training session.

Objective 2: Training is available immediately for selected scientists from Thailand to begin biotechnology-embryo transfer training.

Objective 3: Successful embryo transfer at Mr. Udom Vangtal's dairy would clearly demonstrate that biotechnology-embryo transfer could be accomplished in Thailand. Further, it would demonstrate to other members of the larger dairy cooperatives that improvement in genetic quality of their livestock is possible and can be accomplished within a short time.

Plans for Accomplishing Long-Range Objectives

Personnel

The framework already exists to link scientists interested in biotechnology-embryo transfer from the various universities and research centers. Additional expertise is needed in the animal breeding and dairy management areas to support the milk and beef operations once the genetic potential of the animals is improved.

Thai graduate student training both in Thailand and in the United States or other countries advanced in biotechnology should be considered in the following areas:

- o Introduction of genetic material into mammalian cells and embryos for the improvement of genetic potential of livestock.
- o Cloning of embryos to increase the number of offspring from superior females.
- o Through genetic engineering, development of the ideal dairy and beef animal, pig, sheep, and chicken for maximum production in a tropical environment.
- o Increasing the uniformity of the genetic composition of existing breeds of livestock in Thailand to promote more uniform genetic progress and maximum efficiency.

Facilities

The laboratory facilities located at KURDI are adequate to perform most biotechnology-embryo transfer procedures. Additional micromanipulation equipment is needed to support, for example, the cloning experiments proposed by Dr. Vanda Sujarit.

The Kasetsart University dairy facilities could be improved considerably.

PROPOSED BUDGET

Immediate Objectives

1. Personnel and travel: Travel by two Thai scientists plus per diem to study in the United States for six months. Travel and per deim for two U.S. scientists to visit Thailand to perform embryo transfer and to instruct Thai scientists and veterinarians in embryo transfer.

2. Animal costs: Purchase of 20 embryos from superior U.S. Holstein cows at U.S. $2,250/each.

3. Drug costs: Synchronization and superovulation hormones, culture medium, and minor equipment--U.S. $5,000.

Long-Range Objectives

1. Personnel and travel: Send two Thai Ph.D. scientists to the United States to learn biotechnology skills for 6 to 12 months each. Also, institute a student exchange program to train four Thai students per year in animal biotechnology.

2. Equipment:	Micromanipulator	U.S. $ 35,000
	Sterile room for cell culture	U.S. $200,000
	Supplies for laboratories of Thai scientists	U.S. $150,000/year

3. Additional Travel: Allow travel for Thai scientists to attend scientific meetings to remain current with advancing technologies.

PROPOSED RESEARCH PLAN: SOME SPECIFICS

The advantage to be gained by Thailand entering into the proposed research effort is twofold. First, the potential for improving milk and meat production over current levels is good. Second, the knowledge learned on biotechnology-embryo transfer will be a marketable product to other tropical agricultural countries interested in improving livestock efficiency.

Harvesting Ova

Each ovary is filled with hundreds of thousands of potential ova (eggs) during fetal life, but production of new ova ceases before a female is born. Because there is constant degeneration of ova in the ovary, older animals have fewer ova. In some species about 90 percent of ova degenerate before puberty. More than 99 percent of ova degenerate and fewer than 1 percent ovulate during a female's life, resulting in tremendous wastage. Wastage of sperm is also considerable, but sperm are produced constantly and the nature of the problem is different.

The most common scheme for increasing the harvest of ova is superovulation. Hormones are injected to increase the number of ovulations by a factor of up to 50, but averaging three to eight, depending on the species.

Although superovulation is generally successful, it is exceptionally unreliable for an individual. In cattle, for example, the number of normal ova produced per cow varies from 0 to 50 or more. To date, methods of superovulation have been developed primarily by empirically changing the kind, dose, and timing of administering hormones.

It is possible to harvest ova by mincing the ovary and adding enzymes. However, methods for fertilizing and developing them into normal young are still unknown.

Finally, one of the great unresolved processes in biology is that in which the ovary metes out ova in a controlled way over the reproductive life of females.

Future research should be in the following areas: (1) understanding the physiology of ovum maturation, development, and ovulation so that more rational superovulation regimens can be developed; (2) study of the feedback relationships among the ovary, pituitary, and hypothalamus of the brain; and (3) experiments to obtain ova from the ovary by enzymatic digestion and to grow and develop such ova in the laboratory.

Embryo Recovery and Transfer

Embryo recovery and transfer can be applied successfully to sheep, goats, and swine as well as cattle, but the procedure must be done surgically in these species, and this is expensive and may damage animals. Development of nonsurgical methods for these species is important but not as important as research to develop much better

refinements of embryo transfer and recovery techniques for use in cattle.

Maturation, Fertilization, and Culture of Ova Outside the Body

Maturation of Ova Outside the Body

It is appropriate to emphasize here that considerable work is needed to determine how maturation is controlled. This work will likely result in a real breakthrough in the harvest and use of thousands of ova from a single female.

Fertilization of Ova Outside the Body

Ova harvested in large numbers and matured in the laboratory should be fertilized in the laboratory because fertilization in a living female requires surgical deposition and removal. Furthermore, working out mechanisms of fertilization in the laboratory, where the process can be directed, will provide an understanding of the natural process. As stated earlier, the tools needed to perfect the methods for understanding of fertilization outside the body are now available.

Culture and Development of Embryos

New fertilized ova are not a developmental stage compatible with present technology for frozen storage, sex determination, or nonsurgical transfer. To be useful for embryo transfer, ova fertilized outside the body must undergo development to a state equivalent to that occurring approximately eight days after fertilization in cattle. With present techniques, the proportion of fertilized ova that develop by culture to the eight-day stage is small. In general, the more stages of development the embryos are cultured through, the lower their survival. This is true whether the stages are early or late in embryo development. Research is needed to develop culture systems compatible with maintenance and development of the embryo from fertilized ovum to the early embryo stage. Finally, successful development of ovarian ova will require a combination of highly efficient methods for maturation and fertilization of the ova outside the body and the maturation of most to the early embryo stage.

Cloning and Nuclear Transfer

Sexual reproduction is a random event because eggs and sperm contain only half the genes of the respective parents. The nearly infinite number of possible combinations of genes produces such a variety of progeny that some are likely to survive and reproduce. Others will die without producing offspring.

Animal breeders have tried to improve succeeding generations of animals by removing some of the chance associated with sexual reproduction. Examples of such efforts include formation of breed societies, progeny-testing schemes, amplification of the reproduction of outstanding individuals by artificial insemination and embryo transfer, and establishment of inbred lines to capitalize on uniformity.

Another consequence of the great variation achieved by sexual reproduction is that certain outstanding individuals are produced that satisfy the needs and desires of people to a much greater degree than the average. The curse of sexual reproduction is that progeny of outstanding animals are usually less outstanding than their parents, although they are generally above average. This regression toward the average stems partly from the fact that individuals are a product of genetics and environment and partly from chance when an offspring receives a random half of the genes of each parent.

It is only within the last century that people have had enough information even to dream that they might someday apply technology to produce genetically identical copies of outstanding animals. The ability to clone embryos through several generations of identical clones would provide a tool for accelerating genetic change which is potentially more powerful and effective than artificial insemination. For example, the mating of a proven bull and an outstanding superovulated cow will produce approximately eight embryos. If at the four-cell stage individual cells are removed from the embryo and each again cultured to four cells, 16 embryos would result. If each of these could be broken into four cells and this process repeated six times, 4,096 identical embryos would be produced.

Culture of these embryos to the stage where they could be sexed and frozen would provide over 4,000 embryos of one sex for transfer to recipients. This and other clonal lines from the same superior mating could then be characterized for a production trait, such as milk production, by transfer of a number of frozen embryos from each clonal line to recipient cows to produce heifers to be production tested to evaluate the clone. Once characterized, the embryos from superior clonal lines would be available to create entire herds with the capacity for high production. This would also be a powerful tool for research and genetic improvement.

An alternative and perhaps more immediately feasible approach to the cloning of embryos would be separation of embryo cells at the four- or eight-cell stage before their developmental fate is committed, followed by transfer of their nuclei to fertilized eggs before first cell division. Results of recent research suggest that this is a long-range possibility for development and application of cloning and nuclear transfer technology. However, a vast amount of research is needed to develop the necessary techniques and to determine the limitations of the proposed procedures. In the simplest sense, cloning research starts with the production of twins.

A final method of producing genetically identical mammals is by inserting the nucleus of a body cell into a fertilized egg and then removing or destroying the original genetic complement of the embryo. This already has been accomplished in the mouse, and the procedures were not very different from those used in amphibia.

Embryos from mice and rabbits have been separated into individual cells at the two-, four-, and eight-cell stages, then cultured as single cells back through two, four, and eight cells to the early embryo stage. In general, more survived to become blastocysts when cells were separated at the two-cell stage than at later stages. At best, one-third of the cells at the four-cell stage became embryos. Considerably more research is needed to map out the mechanisms important in embryo manipulation.

Nuclear Transplantation

Replacement of male and female pronuclei in the fertilized mouse egg with a nucleus from a cell of a mouse embryo results in a viable embryo which can develop into a live offspring. Nuclei from cells of an early embryo could be used in nuclear transplantation to form about 100 clones.

The following questions concerning nuclear transfer need to be answered:

- Can live offspring be developed in domestic animals, especially cattle, from embryo nuclear transplantation?
- Is the stage of the cell cycle of donor cells at the time of nuclear transfer critical for continued development of the newly formed clone?
- Is the developmental stage of the pronuclear recipient egg critical for future development of the clone?

Several other exciting technologies of relevance are parthenogenesis, production of chimeras, and development of inbred lines. Parthenogenesis, the production of young without fertilization, occurs naturally in all classes of vertebrates, except mammals. Of particular interest is a strain of turkeys with this characteristic. Only male turkeys are produced in this way, but in mammals only females would be produced. Although parthenogenetic mammalian embryos develop part way through gestation, they do not go to term. Solving this problem would make parthenogenesis extremely useful for some aspects of genetic engineering.

Chimeras are quite valuable for certain experiments. For example, some have certain tissues such as liver derived from one embryo and other tissues such as heart from a second embryo. Furthermore, when parthenogenetic embryos have been mixed with normal embryos, some of the tissues of the resulting adults were derived from the parthenogenetic embryo. As another example, certain cancer cells can be mixed with embryos, resulting in chimeras with perfectly normal tissue derived from the cancer cells, that is, cancer cells changed to normal cells.

Production of inbred animals deserves special mention. Such animals are essentially genetically identical with others in the line, although they may perform poorly due to inbreeding. If two such lines are crossed, however, hybrid vigor results and the animals from such a cross are also genetically identical. Thus the chance element of sexual

reproduction is controlled, resulting in a uniform, predictable product. This is of special value for controlling genetic variation in experimental situations. These are but a few of the questions requiring answers if successful methods for cloning by embryo cell segregation or by nuclear transfer are to be successful. Some tools and basic knowledge to undertake such research are already available.

Storage of Eggs, Sperm, and Embryos

It is often necessary to store sperm, eggs, or embryos for several minutes to several decades. Usually the objective is to maintain these cells in the same state they were in prior to storage, although in some cases it may be desirable to have embryos continue development rather than remain quiescent. Because of varying requirements, storage may be as simple as placing a test tube of embryos on a laboratory bench or as complicated as sequential dilution of semen in a series of complex fluids, followed by freezing to liquid nitrogen temperature in a computer-controlled apparatus.

Successful and inexpensive methods are available for storage of reproductive cells of most species up to several hours. In many cases, storage will also be successful for a day or two, but techniques are more complex and sometimes less effective than for shorter storage. For storage more than several days, the method of choice is freezing to liquid nitrogen temperature.

Adequate methods are already available for freezing bull and turkey semen; however, there is room for improvement. About half of the sperm are killed in the process and others damaged so that three times more frozen sperm are required per insemination to obtain fertility than unfrozen semen. Furthermore, semen from some individual animals cannot be frozen successfully at all.

In general, good methods for freezing semen of sheep, goats, swine, and chickens are not available. Recent research led to considerable improvements with these species, but much remains to be done.

Relatively little research has been done with freezing ova, but this may be feasible. There has been considerable experimentation with freezing embryos. This is not yet possible with swine, but embryos from sheep, goats, and cattle can be frozen, although at a cost of killing half of them. Even with a 50 percent loss, this technology is already being used commercially for certain situations. There is great demand for frozen bovine embryos, and this technology will be used widely if losses from freezing can be lowered from 50 percent to 20 percent.

The major constraints in freezing reproductive cells are lack of basic knowledge concerning mechanisms of cell injury, how cryoprotectants work, and why cells from certain individual animals and species are so susceptible to freezing damage. Although strides have been great in developing a theoretical groundwork for freezing technology in recent years, much remains to be done so that we can move from the current phase of trial-and-error testing of infinite combinations of freezing rates, thawing rates, cryoprotectants, buffers, etc., to a more directable strategy. Further research should include: (1) basic research on

how and why cells respond as they do to cryoprotectants, freezing, and thawing; and (2) continuation of the empirical approach using clues from basic research to determine which factors should be studied.

The benefits of storing sperm, oocytes, and embryos at liquid nitrogen temperatures are enormous, both from commercial and research perspectives. Such storage greatly increases flexibility because the subjects need not be synchronized precisely in time and space. This leads to tremendous savings in manpower and feed costs.

Sex Selection: Sperm Fractionation

There is no repeatably successful procedure in any species for separating sperm that determine male offspring (Y-bearing sperm) from those that determine female offspring (X-bearing sperm). Efforts have appeared promising in the past (for a review, see Sex Ratio at Birth--Prospects for Control (Kiddy and Hafs, 1970), and will continue to excite scientific inquiry. However, in the most recent review of literature on sex determination and differentiation (Hawk, 1979), R. E. Short concludes, "And the tantalizing tale of artificial regulation of the primary sex ration remains a mirage on the horizon."

In many birds and mammals there is a distinct difference between the sexes in morphology of chromosomes. In mammals, females have two X chromosomes and males one X and one Y chromosome. Theoretically, one needs to observe the chromosomes in only one cell of an animal or embryo to determine its sex. A review of this methodology has been provided by Hare and Betteridge (1978).

The current state of the art is such that embryos can be divided with greater than 95 percent accuracy into three approximately equal groups by sex chromosomes: male, female, and unknown. Pregnancy rates following transfer of sexed embryos is reduced slightly. Nearly a full day is consumed from the time an embryo is biopsied until its sex has been determined. Before this technology can be applied commercially, it must be made simple, fast, inexpensive, reliable, and safe for embryos.

A more sophisticated method of sexing is to observe whether male- or female-specific proteins (or RNA) are being produced. One such protein produced by all male cells and not by female cells is the H-Y antigen (Ohno, 1979). This protein appears to be coded by DNA on the Y chromosome, and thousands of copies are inserted into each male cell membrane. Because so many molecules are in the cell, detection of the protein might be a more sensitive assay than direct observation of the DNA, of which there is only one copy. The presence of the protein is detected by antibodies. A method for sexing embryos with anti-H-Y antibody and a fluorescence technique is in the preliminary stages of development. It may enable sex determination without the need to kill or biopsy the embryo, although a few cells may die as a result.

The attractiveness of sex control by separation of X- and Y-bearing sperm dictates that further research be devoted to this problem as new leads become available. Experiments should continue on improving the sexing of embryos by sex chromosome morphology, with probably a major

emphasis on detection of gene products of the Y chromosome such as H-Y antigen. Production of monoclonal antibodies to H-Y and use of such antibodies to sex embryos are examples of what might be done.

Twinning by Embryo Transfer

Nearly 80 percent of the cows in North America are beef cows with the sole function of producing a calf each year. Approximately 70 percent of the nutrients consumed by each beef cow are for her own maintenance, whereas only about 30 percent are for growth and maintenance of the calf during pregnancy and lactation. The low reproductive rate of cattle--the greatest limitation in converting forage to meat--would be ameliorated if cows had twins. The approach offering the most hope for greatly improved meat-producing efficiency in cattle is the induction of twinning by embryo transfer. Its successful development and implementation would be a major breakthrough in the technology of food production.

Currently, embryo transfer is the most effective method of inducing twin pregnancies in cattle. When twinning is induced, pregnancy rates range between 67 and 91 percent. Between 27 and 75 percent of pregnant cows deliver twins. The most cost-efficient method for achieving this is to transfer an embryo to a heifer that was inseminated at estrus. Pregnancy and twinning rates have been higher when two embryos were transferred to cows that had not been inseminated. This was attributed to low fertility following artificial insemination in the group that received a single embryo. Hormonal induction of twinning, a modification of superovulation, has not been developed into a reliable procedure. Even if the technology were available, it would not be the best method since both ovulations might be on the same ovary.

IMPACT OF RESEARCH

The potential impacts and long-range payoff for the research described as embryo technology are enormous. With the current technology success rate, embryo transfer could lead to an increase in the rate of genetic improvement of from 10 percent to nearly 100 percent, depending principally on current intensity of sire selection and on the trait(s) in question. Van Vleck (1980) has estimated the possible effects of alternative technologies on the rate of genetic improvement in milk yield in dairy cattle in kilograms/cow/year: regular artificial insemination, 100; sexed semen, 115; embryo transfer, 158; and sexed semen and embryo transfer, 166. He has also shown that cloning, if available, could be used to increase accuracy of genetic evaluation of dairy cattle over that possible from sister and progeny tests. The accuracy is doubled under conditions giving maximum advantages to cloning and is about one-third to one-half higher under more realistic assumptions.

These technologies have important applications in addition to increasing rate of genetic improvement. Sexed semen, for example, has

economic implications. Based on the estimate that a male can produce 10 percent more lean beef than a female on a given amount of feed, the reduction in the proportion of females from the present 50 percent to the approximately 20 percent needed to maintain the beef cattle breeding herd would increase total beef production per unit of feed by 3 percent. Embryo transfer also has important economic applications in multiplying rare breeds, strains, or individuals, and, in conjunction with the use of frozen embryos, in avoiding the high costs of animal shipment and quarantine incurred in transferring livestock breeds to new environments for either production or research.

Some of the most important applications of these technologies are in research in other disciplines of animal science. Identical twins have been used to provide genetic uniformity for nutrition and other research, but their use is severely limited by their scarcity and the cost of proving identify. The availability of clones would reduce the numbers of animals needed for specified precision by an estimated 20-50 percent in many livestock experiments.

Embryo transfer is a powerful tool for separating contributions of maternal and offspring genes to prenatal survival and growth in mammals. This technology has been used effectively to measure uterine capacity and genetic control of the initiation of parturition. The techniques has many potential applications to development of new knowledge of uterine function and interactions between the embryo and uterus.

The economic benefits from increasing the reproductive efficiency of food-producing animals are potentially enormous. Investigators have calculated the benefits that dairy cattle and beef cattle would derive from the application of technology in (1) estrus, ovulation, and fertilization; (2) embryo survival and development; and (3) parturition and perinatal survival:

- o Dairy cattle. The dairy cow population in Thailand is some 60,000 head. By controlling the onset of estrus and ovulation earlier in the postpartum period through the approaches described and by improving automation of estrus detection, the calving interval could be reduced from 18 months to 12 months. The poorest 40 percent of the cows could be marketed, and the remaining 60 percent would provide the current milk supply. This would yield savings on seed costs of 336 million baht* and an additional cost savings for labor.

- o Beef cattle. The calf crop, calculated as a percentage of cows exposed to bulls, could be increased from 50 to 75 percent by increased pregnancy rates and reduced calf mortality. For example, an increase in the beef calf crop of the 1981 Thailand beef herd, 1981, of 5.6 million (1981 FAO Yearbook) of from 50 to 75 percent--taking into account maintenance requirements per cow of 1,000 kg total digestible nutrients (TDN), feed costs of 730 baht/ton pasture dry basis for 12 months, and nonfeed costs of 460 baht/cow would have meant reducing the cow herd by 2.8 million head and reducing the feed and maintenance requirements per cow by 460 baht. The total savings would have been 170 million baht/year.

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*60,000 cows x 40 percent reduction x 46 baht/day/cow x 305 day lactation.

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5. PLANT CELL CULTURE AND ALTERNATIVE CROPS

INTRODUCTION

Plant cell culture techniques cover several technologies, including clonal propagation, tissue culture-induced variation (somaclonal variation), anther culture, protoplast fusion, and recombinant DNA gene transfer. These technologies are listed from the easiest to the most difficult. Any program in plant cell culture should use one or more of these techniques to make a significant improvement in one or more crops. Thai scientists have had success in applying the easiest of these techniques, clonal propagation, toward improvement of their cut flower industry. They have not successfully applied any of the other technologies.

It is important to realize that each technology offers some unique opportunities not afforded by the other techniques. Scientists at the U.S.-based DNA Plant Technology Corporation (DNAP), who have internationally recognized experts in each of these areas, have been involved in the development and commercial application of all five of these technologies.

GENERAL KNOWLEDGE

Worldwide

There has been a tremendous explosion of interest in plant cell culture during the past five to 10 years. Several laboratories in both developing and developed countries have been utilizing clonal propagation for production of genetically uniform plants, particularly ornamentals. In the United States, this approach has been restricted by high labor costs, and cloning is now used only for orchids, azaleas, and some cut flowers, such as gerberas. The technique used is quite simple--a rapid-growing meristem or seed-derived embryo is placed onto medium that is suitable for rapid shoot proliferation. Shoots are separated and placed onto rooting medium. Young plants are slowly acclimated to greenhouse and then field conditions. This technique is simple, albeit labor-intensive.

In the past two to three years, several laboratories have been interested in the use of tissue culture to select and identify new variants. Researchers have used this technology to develop new disease resistant clones of sugarcane which are currently being grown in Taiwan and Australia. U.S. scientists have used this technology to develop new breeding lines in several crops as well as a new variety of tomato. Based on their research in tomato, they have gained considerable knowledge of how to apply this technology to several other crops. Somaclonal variation must be combined with conventional breeding to develop new varieties. For this reason, very few laboratories have successfully used this technology. However, since the techniques are quite simple, a properly managed program would have a very high probability of success.

Protoplast fusion has been explored in recent years as a means of achieving new gene combinations that are not possible using conventional breeding. Many very distant hybrids may be sterile or unstable, but many combinations have recently resulted in new breeding lines. U.S. scientists were able to successfully transfer disease resistance and insect resistance using protoplast fusion.

Anther culture--recovery of plants from the male reproductive structures--can be used to recover new true breeding lines for the development of new varieties. This technique is particularly appealing for developing lines from "hybrids," where it would be possible to construct new true breeding lines that have the best characteristics of the hybrid. The Chinese have used this technique to develop new varieties of rice, wheat, and tobacco. It is imperative that anther culture be linked to a breeding program.

Finally, recombinant DNA has been touted as the ultimate method for transferring useful traits into existing cultivars. To date, no agriculturally useful trait has been transferred into any cultivated crop using recombinant DNA. Hence, the technology is 10-15 years away from routine commercial application.

Thailand

Scientists in Bangkok and Chiang Mai have experience with clonal propagation. Cell culture is being used for commercial propagation of orchids in Bangkok. University laboratories have developed cloning methods for orchids, gerberas, potato, gladiolus, carnation, and teak. The teak cloning method is particularly exciting as it has been used to reduce the time to harvest from 60 years to 40 years. This work in Chiang Mai should definitely be nurtured. When the Thai refer to tissue culture they almost always mean clonal propagation--the simplest of the five technologies.

The limited work initiated on somaclonal variation, under the direction of Montakan Vajrabhaya at Chulalongkorn University, is currently sponsored by a grant from USAID and appears to be proceeding nicely. The aim is to use cell culture to develop salt-tolerant rice.

For protoplast fusion, anther culture, and recombinant DNA, there is virtually no expertise in Thailand. These technologies will have to be imported into Thailand if they are to be used.

CAPABILITY

Human Resources

Several scientists in Thailand have been trained overseas in plant tissue culture; however, none have received training in anther culture, protoplast fusion, somaclonal variation, or recombinant DNA. Nearly all of the tissue culturists have been trained for micropropagation (cloning) via tissue culture. While this has been suitable for the cloning of ornamentals, it will be insufficient for biotechnological development using the other technologies. It would be most useful for two to three people to receive training from a single or small number of laboratories in the United States to overcome this deficiency.

Facilities

Plant tissue culture does not require a large amount of equipment. Several of the laboratories need laminar flow hoods, inverted microscopes, and growth chambers. In the United States, this would cost $20,000 per laboratory.

CONSTRAINTS

Currently, two factors limit immediate use of tissue culture to solve Thai agriculture problems:

- Limited training of scientific staff. No scientists are actively using several key technologies.
- Funding and scientific interaction. Funding is currently limited for crop plant improvement, hence, little work is done in this area, and there is limited coordination of disciplines needed for solving crop problems.

TIME REQUIRED TO SOLVE THE PROBLEMS

Several problems with annual crops could be solved in three years. With an increase in the amount of seed, new improved varieties could be released, using tissue culture, within five years or less. Some problems can be solved using short-term approaches while longer-term techniques are being developed.

OVERALL RECOMMENDATIONS

Based on discussions with the Thai committee members, the following recommendations are made:

- o A committee of existing plant tissue culturists should be formed and should meet at least every two months to discuss research projects.
- o Monetary support for existing laboratories should be increased. Support should be given to encourage researchers to work on major food crops. A grants program should be instituted for existing laboratories.
- o Arrangements should be made to obtain additional training overseas for several Thai scientists who are currently trained in plant micropropagation.
- o A substantial program (three years minimum and five years optimum) that includes parallel research should be established in the United States for a major food crop, rice, cassava, or maize. Work should concentrate on application of anther culture, protoplast fusion, or somaclonal variation for crop improvement. Funding could be sought from USAID or another international funding agency. Once success is achieved in the United States, these technologies could be transferred to Thailand. This is also a program that would be of interest to a U.S. company. (For example, the U.S. company could be involved in training several scientists in new technology areas applied to maize, cassava, rice, potato, etc., while also working on these crops for Thailand.)

SPECIAL PROBLEMS OR OPPORTUNITIES

Potato Seed

Potatoes are grown in the highlands and could be grown more extensively if the quality of the product was improved. Unfortunately, nearly all of the potatoes grown must be imported as fibers from The Netherlands, requiring substantial expenditures overseas for seed material. Efforts are being made in Chiang Mai (Department of Plant Pathology) to develop virus-free stock plants of potato. If propagated, these could produce tubers suitable for establishment of new crops. However, once the potatoes are transferred to the field, they could become reinfected. To obtain new materials for planting, the "seed" potato must be grown in uninfected soils, so that even if the Chiang Mai program is successful, it is likely that potato tubers will still be imported.

One solution to this problem is the use of plant tissue culture for in vitro tuberization. For this reason, the best germ plasm are identified and cloned. Shoots are then induced to form small tubers in vitro which are used in turn to establish field plantings. As they are tubers, they can be stored for long periods of time and, most important, they will be free of virus. This same approach could be used for nearly all tuber crops, such as yams.

Technical and Commercial Feasibility

This technique is currently being used in the United States to supply potato farmers in Idaho with virus-free potato tubers. It is both technically feasible and commercially viable. A market study should be completed to determine the size of the market in Thailand and in Asia for export. For example, such tubers may be particularly valuable for export to Korea.

Highland Vegetables and Heat Tolerant Vegetables

Tomatoes, eggplant, carrots, celery, and similar plants are all high-value crops. In Thailand, these vegetables grow best in the highland areas, which, unfortunately, have constant rain and cool evening temperatures, conditions that are perfect for fungal infection. Thus, tomato plants and potatoes suffer from Atternaria, Phythophthora, Rhizoctonia, etc. These pests are all present in the rainy climate and some (e.g., Phythophthora--late blight in tomato) cause considerable damage. It is possible to use existing technologies of somaclonal variation, anther culture, and protoplast fusion to transfer disease resistance into these vegetables. Alternatively, the introduction of heat and moisture tolerance into some varieties would permit their cultivation in the lowlands.

Technical and Commercial Feasibility

Thailand has only limited land suitable for the growth of vegetables; thus, existing markets are not large. With the development of varieties suitable for Thailand, however, this market would expand considerably. Tomatoes, for example, could be exported to many areas of Southeast Asia.

Alternatives

Conventional breeding could be applied to achieve similar results but would require seven to eight years to produce a new variety with disease resistance. The time savings using somaclonal variation is considerable--three to four years for release of a new variety.

High-Yielding Cassava, Improved Rice Varieties, and Sugarcane Varieties

Present yields of cassava in Thailand are making it an unprofitable crop. The achievement of greater yields of cassava will combat its low unit value and make it more suitable for biomass conversion.

Cassava varietal improvement would benefit greatly from cellular genetic methods since problems of disease or virus resistance, and yield

and composition of biomass can be modified using somaclonal variation, protoplast fusion, and anther culture. In addition, since both cassava and sugarcane are asexually propagated, there is no problem in establishing new fields of superior crops.

A biotechnology program should be in place for each of the major crops and exports of Thailand--rice, sugarcane, and cassava. Without such a program, it is unlikely that Thailand can keep up with the falling world market price, and with the spread of new diseases.

Technical and Commercial Feasibility

This program would most likely not be attractive to private industry since what is proposed resembles more an insurance policy than a new profit opportunity. Somaclonal variation has already been used to develop new disease-resistant varieties of sugarcane, anther culture to produce new varieties of rice in China, and tissue culture to eliminate virus from cassava. Thus, tissue culture has been used for each of these crops, although none of them is easy to grow in cell culture. It would be necessary to train Thai scientists in cell culture approaches to each of these crops. One program on development of salt-tolerant rice is already in place in Bangkok.

Alternatives

Conventional breeding programs could be established to support each of these crops, but each program will require an extended period of time. Thus codevelopment of breeding and biotechnology programs is appropriate.

Finally, it should be noted that if any of these crops, such as cassava, mesta, or sugarcane, is used for a biomass program, the implementation of a biotechnology program would permit greater flexibility in development of new crop varieties ideally suited for biomass conversion. In Brazil, for example, cell culture (somaclonal variation) is being used to develop new varieties better suited to ethanol production.

Maize Improvement

The fourth major crop of Thailand is maize, which is grown mainly for animal feed. The present outlook for maize is not good due to low yields and aflatoxin contamination, and its value has been decreasing. Attention should be directed toward development of maize breeding lines more ideally suited to Thailand and opening new markets for maize.

Maize can be grown for starch production, for human consumption (sweet or baby corn), or for animal feed. A cell culture program that includes the use of somaclonal variation, protoplast fusion, and anther culture to develop new varieties could make Thailand a world leader in the development of new maize varieties. Such a program should be backed up by a program in a U.S. corporation to aid in germ plasm collection

and technology development and training. For example, DNAP has developed procedures for plant regeneration of maize using somaclonal variation and anther culture.

The program described here could be used to develop aflatoxin-resistant maize, high-yielding, high-protein maize (starting with opaque varieties), disease-resistant sweet corn, higher-yielding baby corn for export, and new popcorn varieties.

Technical and Commercial Feasibility

Corn is more difficult to work with in cell culture than the other crops mentioned here. There are no programs in corn tissue culture in Thailand, despite corn being the number four crop. Thus, a back-up program, initially in the United States, is necessary to develop and transfer technology to Thailand. Perhaps this could be accomplished using USAID funds.

A U.S. corporation should be interested in this type of program as it has a high likelihood of success and a high probability of commercialization.

Alternatives

The only alternative is to continue to rely on germ plasm that has been uniquely selected for performance in the Western Hemisphere by breeders with different interests.

OVERALL EVALUATION

Thai scientists are well trained in clonal propagation. Unfortunately, no laboratory is successfully applying any of the technologies listed in the introduction to this report. It is recommended that Thai scientists receive immediate training and greatly increased levels of funding to enter into this important area. With wise investment, biotechnology can be used to make significant improvement in Thailand's crops.

APPENDIXES

APPENDIX A

MEMBERS OF ADVISORY AND WORKING GROUPS

Advisory Group on Organization of Center

Dr. Bhichit Rattakul, Vice-Director, National Center for Genetic Engineering and Biotechnology, Thailand

Dr. Richard Herrett, Director of Research and Development, ICI Americas, Inc., Goldsboro, North Carolina

Dr. Hugh Popenoe, Director, International Programs in Agriculture, and Director, Center for Tropical Agriculture, University of Florida, Gainesville, Florida (Overall Coordinator, U.S. group)

Dr. Malee Suwana-adth, Director, National Center for Genetic Engineering and Biotechnology, Ministry of Science, Technology and Energy, Royal Government of Thailand

Dr. Yongyuth Yuthavong, Vice-Director, National Center for Genetic Engineering and Biotechnology, Thailand

Mrs. Rose Bannigan, Program Development Coordinator, Board on Science and Technology for International Development, Office of International Affairs, National Research Council, Washington, D.C. (Staff Coordinating Officer, U.S. group)

Working Group on Diseases of Cultured Freshwater Fish in Thailand

Dr. John L. Fryer, Professor and Chairman, Microbiology Department, Oregon Oregon State University, Corvallis, Oregon

Mrs. Kamolporn Pavaputanon, National Inland Fisheries Institute, Thailand

Dr. Kriangsuk Saitanoo, Chulalongkorn University, Thailand

Dr. Malinee Limpoka, Kasetsart University, Thailand

Dr. Thanit Kusamran, Mahidol University, Thailand

Dr. Thirapan Bhukasawan, National Inland Fisheries Institute, Thailand

Working Group on Aflatoxin in Thai Corn

Dr. Chalermlarb Chuaiprasit, Pathologist, Department of Plant Pathology, Kasetsart University, Thailand

Ms. Dara Buangsuwan, Department of Agriculture, Thailand

Dr. Pitak Chirapinyo, Corn Association, Thailand

Dr. Urban Diener, Professor, Department of Botany, Plant Pathology, and Microbiology, Auburn University, Alabama

Ms. Supatra Mansakul, Thailand Institute of Scientific and Technological Research, Thailand

Dr. Sutat Sriwatanapongse, Corn Breeder, Department of Agronomy, Kasetsart University, Thailand

Dr. Thirayuth Glinsukon, Mahidol University, Thailand

Working Group on Utilization of Cassava

Dr. Busaba Yongsamith, Kasetsart University, Thailand

Dr. George E. Inglett, Chief, Cereal Science and Food Laboratory, Agriculture Research Center, U.S. Department of Agriculture, Peoria, Illinois

Mrs. Jiraporn Sukhumavasi, Thailand

Dr. Malee Suwana-adth, Director, NCGEB, Thailand

Mrs. Napha Lotong, Thailand

Dr. Pairoh Pinpanitchakan, Chulalongkorn University, Thailand

Mr. Parinya Amornsirisomboon, Thailand

Dr. Pivan Varangoon, TISTR, Thailand

Mrs. Suchai Wong-ngam-nit, Thailand

Mrs. Sunanta Ramanvongse, TISTR, Thailand

Working Group on the Development of Animal Biotechnology in Thailand

Mr. Chamnien Satayapant, Kasetsart University, Thailand

Dr. Charan Chantalakhana, Kasetsart University, Thailand

Dr. Kamphol Adylavidhaya, Director, KURDI, Kasetsart University, Thailand

Dr. Maneewan Kamolpatana, Chulalongkorn University, Thailand

Dr. Nati Nilnophakoon, Faculty of Veterinary Medicine, Kasetsart University, Thailand

Mr. Udom Wangtan, Nongpo Cooperative, Thailand

Dr. Vanda Sujarit, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Thailand

Dr. Wanapa Sucharit, Kasetsart University, Thailand

Dr. Raymond W. Wright, Jr., Professor of Cell Biology and Animal Scientist Department of Animal Sciences, Washington State University, Pullman

Working Group for Plant Cell Culture and Alternative Crops

Dr. David Evans, Associate Director for Research, DNA Plant Technology Corporation, Cinnaminson, New Jersey

Dr. Montakan Vajrabhaya, Chulalongkorn University, Thailand

Dr. Paiboolya Kavinlertvatana, Kasetsart University, Thailand

Dr. Pimchai Apawacharut, Chiangmai University, Thailand

Dr. Siriporn Nitayangkul, Mahidol University, Thailand

APPENDIX B

Institutions and Manpower Engaged in Biotechnology and Genetic Engineering in Thailand

INSTITUTION	LOCATION	CORE STRENGTH*
Mahidol University		
Faculty of Science (including Center for Biotechnology and Center for Molecular Genetics and Genetic Engineering)	Bangkok	40
Institute of Nutrition Nakorn Pathom	Bangkok and	20
Faculty of Medicine, Siriraj Hospital (including Center for Thalassaemia)	Bangkok	10
Faculty of Medicine, Ramathibodi Hospital	Bangkok	10
Faculty of Pharmacy	Bangkok	5
Chulalongkorn University		
Faculty of Science (including Institute for Biotechnology and Genetic Engineering)	Bangkok	30
Faculty of Engineering	Bangkok	5
Kasetsart University	Bangkok and Nakorn Pathom	30
Institute of Food Research and Product Development	Bangkok	10
King Mongkut's Institute of Technology, Thonburi campus	Thonburi	20
Thailand Institute of Scientific and Technology Research (TISTR)	Bangkok	20

APPENDIX B (continued)

Asian Institute of Technology	Bangkok	10
Chiang Mai University	Chiang Mai	10
Khon Kaen University	Khon Kaen	5
Prince of Songkla University	Songkla	5
Department of Agriculture	Bangkok	30
The Thai Red Cross	Bangkok	10

*R&D personnel with M.Sc. qualification or higher working on biotechnology and genetic engineering.

APPENDIX C

BIOTECHNOLOGY PROGRAMS AND PROJECTS AT MAJOR INSTITUTIONS IN THAILAND

Four major universities and the Thailand Institute for Scientific and Technological Research (TISTR) have various activities in biotechnology and genetic engineering and are in various stages of institutionalizing their programs. Their activities are outlined below.

MAHIDOL UNIVERSITY

This university has a long-established international reputation in medical and basic bioscience. This is partly due to a successful joint 10-year program with the Rockefeller Foundation launched in the early 1960s for development of graduate study and research. Mahidol University is still by far the most important institution in various areas of bioscience, supplying Ph.D. and M.Sc. graduates to staff other universities and institutions both in Thailand and neighboring countries. In 1979, the university created the Center for Biotechnology and the Center for Molecular Genetics and Genetic Engineering to concentrate on the rapidly emerging fields at the level of research and development. It also recently launched an undergraduate program in biotechnology. Current major research interests include:

- Diagnosis and characterization of malaria parasites and other pathogens. Characterization of vectors of tropical diseases. Diagnosis of major genetic diseases (thalassaemia, abnormal hemoglobins, etc.).
- Development of vaccines for dengue, hemorrhagic fever, hepatitis, and other infectious diseases.
- Development and production of bacteria and their toxins which kill larvae of mosquitoes and other insect pests.
- Development of new agents and processes for control of human reproduction.
- Detection and toxicology of mycotoxins commonly found in food and other agricultural products.
- Isolation and development of new antibiotics and other drugs from microorganisms and plants indigenous to Thailand.
- Improvement of local fermentation processes for production of foods and food seasonings, with emphasis on amino acid production.

- o Field testing of selected lignocellulolytic fungi for local production of fodder from rice straw and water hyacinth.
- o Modification of natural rubber for improved properties and selective purposes.

CHULALONGKORN UNIVERSITY

The university started a graduate program in biotechnology in 1982, and in the following year established the Institute for Biotechnology and Genetic Engineering. These programs conduct research and development intended to contribute to small- and medium-scale industry for production of biochemicals. Major research interests include:

- o Bioconversion of starch to glucose, fructose, sorbitol, etc.
- o Production of selected enzymes, e.g., penicillin acylase, glucose isomerase, amylase, glucoamylase, and bromelain.
- o Use of immobilized penicillin acylase for production of aminopenicillic acid, a key intermediate in antibiotics production.
- o Improvement of nitrogen fixation in microorganisms.

KASETSART UNIVERSITY

The Department of Biotechnology at Kasetsart University currently runs an undergraduate program in biotechnology, and its Institute of Food Research and Product Development is undertaking many R&D projects, especially on ethanol fermentation. The university has a new campus at Kamphaengsaen, Nakorn Pathom, where field experiments are carried out in various aspects of agricultural biotechnology. Major research interests include:

- o Improvement of ethanol production from cassava starch.
- o Production of amylase, glucoamylase, and other industrially useful enzymes from improved strains of microorganisms.
- o Immobilization and utilization of enzymes in the production of food products.
- o Biological nitrogen fixation for improved crop yields.
- o Tissue culture for improvement of economic crops, including orchids and sugarcane.
- o Biotechnology of aquaculture and mariculture.
- o Optimal biogas production from agricultural and industrial waste and by-products.

KING MONGKUT'S INSTITUTE OF TECHNOLOGY, THONBURI CAMPUS

The Thonburi Campus has set up an R&D Operation Center in which biotechnology plays a part. The campus is strong in process engineering and other engineering aspects of biotechnology. Pilot plants and some

experimental equipment are homemade and developed to suit local conditions. Research interests include:

- o Use of improved microbial strains in nitrogen fixation.
- o Production of biogas from agricultural wastes and by-products on an industrial scale.
- o Design and development of pilot plants for alcohol fermentation and other processes.
- o Development of enzymatic membrane reactors.

THAILAND INSTITUTE FOR SCIENTIFIC AND TECHNOLOGICAL RESEARCH

This broad-based R&D institute is the only industrial institute in Thailand without any university links. Biotechnology activities are related to the institute's work, ranging from food and energy to pharmaceuticals. TISTR recently completed a pilot plant with a production capacity of 1,500 liters per day, and is conducting a cooperative project with the Japanese Association of Industrial Fermentation on pilot-scale production of power alcohol from cassava starch. Other activities include operating the Microbial Resources Center for Southeast Asian Region (supported by the United Nations Environment Programme/United Nationsl Educational, Scientific and Cultural Organization/International Cell Research Organization Panel on Microbiology) and the National Gene Bank of Thailand (supported by the International Board for Plant Genetic Resources).

APPENDIX D

SUGGESTED PROPOSAL FORMAT FOR INDIVIDUAL GRANTS

A proper project plan should include the following elements:

Abstract. A summary of about 200 words describing the proposed research work and its relationship to the general problem area. This summary should be informative for other scientists in the same or related fields, yet understandable by laymen. It should include the total amount of funds requested or needed and the proposed duration of the project.

Background. A brief technical description of the subject that is understandable to other scientists and that includes discussion of the scientific and technological background and its relation to the main problem area. It should include baseline data to define surrounding conditions and facets of the problem as well as a review of literature on the state of the art, both domestic and foreign. The views and findings of peers in other universities or ministries working in similar fields in Thailand should be reflected to assure an adequate picture of the current situation.

Impact Statement. This is a quantitative statement of the anticipated benefits of the project relative to baseline conditions and includes both the immediate results and the long-term benefits following implementation of the solution. This statement should include, whenever possible, quantifiable or measurable elements as they are helpful in describing anticipated or potential impacts. Implicit in the impact statement is a definition of the long-term goals toward which the project is expected to contribute.

Objective. A clear, concise, detailed statement of the project objective, including:

- o General (related to project goals)
- o Specific objectives of the proposed research in verifiable terms.

Research Plan. The heart of a proposal, which should be directed to specialists in the field. For each phase of the project, the following should be provided:

- o Hypothesis to be tested

- o Methodologies to be tested
- o Activities to be carried out
- o Inputs required
- o Timetable and duration of this phase
- o Ethical considerations if human subjects are to be used in the research
- o Resources.

In the resources section, two very important aspects should be considered: manpower and organization and facilities. The manpower and organization subsection describes how the project group will be structured and organized and its reporting channels. It also outlines the subprojects and tasks for all individuals or groups involved so that individual responsibilities are clear as well as the time or the fraction of time required for each task. If certain skills are not available in-house, arrangements for obtaining them elsewhere or collaborating with another group or groups must be clearly defined. Realistic assignments must be based on the actual time that a specialist can devote to a project. Each task and the manpower and time required to accomplish it should be estimated with the participation of the individual who will do the work.

The subsection on facilities clearly states where the project will be conducted and what equipment or facilities are required. In some cases, opportunities for sharing with other organizations must be explored and the necessary arrangements made. If special equipment is to be procured, a statement of cost is included as well as a reasonable time schedule for its order, delivery, installation, and calibration at the project. Since long lead times may be needed to obtain the appropriate equipment, planning must be carefully coordinated with manpower needs so that operators, for example, are not scheduled until actually needed.

Time Schedule and Milestones. The schedule for the overall project shows the beginning and end of individual tasks and establishes appropriate project milestones. Milestones are points in time when major technical results should appear, when equipment should be ready for service, or when critical decisions should be made. Milestones also indicate when an overall project evaluation or assessment is to be made so that mid-course corrections can be applied if necessary. Properly developed, the time schedule permits one to judge the effectiveness of project management. Its quality contributes to the credibility of the total plan.

Budget. The budget lists the total expense to the organization of conducting the project, and it includes routine expenses (salaries and overhead, if appropriate) as well as development expenses. The budget is compiled and displayed on a task-by-task basis, so that both financial and technical progress can be reviewed during project evaluations. Properly compiled, the budget permits one to judge the effectiveness of project management.