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GENETIC ENGINEERING AND BIOTECHNOLOGY MONITOR

Vol.1, Nos. 1 & 2, 1994 (Double issue)

CONTENTS

UNIDO's *Genetic Engineering and Biotechnology Monitor* is established as a mechanism of current awareness to monitor developments in the gene ic engineering and biotechnology sector and inform governments, industry and academia, primarily in developing countries

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TO OUR READERS

The Inter-Agency Committee on Sustainable Development (IACD), at its second session, approved the responsibilities and functions of the Task Managers for the various chapters, issues and programme areas of Agenda 21, which arose from the UN Conference on Environment and Development held in Rio de Janeiro in 1992. The main functions of the Task Managers are to promote information exchange and interagency contact, catalyze joint activities and programmes, and develop common strategies to implement relevant parts of Agenda 21. In addition, it has been agreed that the Task Managers will, in collaboration with the organizations concerned, prepare coordinated inputs for the consolidated report of the Secretary-General, that will focus on common UN System strategies for the implementation of Agenda 21 and identify areas for further action for consideration by the Commission on Sustainable Development.

Agenda 21 addresses the pressing problems of today and aims at preparing the world for the challenges of the next century. It reflects a global consensus and political commitment at the highest level on development and environment cooperation. Agenda 21 is a dynamic programme and will be carried out by the various actors according to the different situations, capacities and priorities of countries and regions in full respect of all the principles contained in the Rio Declaration on Environment and Development. It could evolve in the course of time, in the light of changing needs and circumstances.

Since UNIDO has been designated the Task Manager on Chapter 16 of Agenda 21 (Environmentally Sound Management of Biotechnology) we will welcome contributions from readers of the *Monitor* who are actively involved in promoting sustainability in the field of biotechnology. An *ad hoc* UN interagency consultative meeting to prepare the scope of and procedure for the preparation of the Task Manager's Report on Chapter 16 is planned to be held in mid-September, for which position papers sent to us by readers from biotechnology industry associations, non-governmental organizations, research policy establishments and the like, may be taken into consideration for background documentation to the report. To this end you will find in this issue a loose leaf sheet with points for consideration, but this is strictly only to be viewed as a study guide.

You will nctice we have a newly designed cover – the next issue will have slightly different print A time for change, hopefully for the better.

Malee Suwana-Adth
Scientific Editor

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A. NEWS AND EVENTS

UNIDO news

UNIDO's biotechnology programmes for Africa

As part of its programme of offering opportunities to African countries in a comprehensive way for sustainable economic and social development, a set of initiatives involving biotechnology applications that can positively impact important sectors and yet operate at a level appropriate to each country has been prepared. Biotechnologies can play essential roles in fostering development, and will give competitive advantage to nations that harness its possibilities. UNIDO's programme operates both vertically, in fostering development in step-wise fashion from simple to more sophisticated as countries develop infrastructural support in biotechnology expertise, and horizontally, or regionally, as networks are built and developed on themes of common interest.

I. Assisting African countries to acquire infrastructural expertise and support for implementing initiatives in biotechnology

In December 1993, UNIDO sponsored an African Regional Training Course on Modern Biotechnologies in Harare, Zimbabwe. The International Centre for Genetic Engineering and Biotechnology (ICGEB) and the Intermediary Biotechnology Service (IBS) at the International Service for National Agricultural Research (ISNAR) each contributed a resource person to the course. It is UNIDO's intention to develop, in collaboration with IBS at ISNAR, the facilities at the University of Zimbabwe to become a regional training centre within Africa for the training of African scientists in these advanced biotechnologies.

The ICGEB has been a UNIDO project since its initiation in 1983; it became operational in 1986, and has been the only operating laboratory in the field of genetic engineering within the United Nations system. The ICGEB is to become an autonomous international agency in early 1994, but a continuing close association between the ICGEB and UNIDO presents a unique opportunity for ongoing promotion of fundamental and applied research in biotechnologies for the benefit of developing countries in support of their industrial development.

II. Regional support for biotechnological development

The African Regional Centre for Technology (ARCT) and UNIDO have had a cooperative agreement to work together for some years. In conjunction with UNIDO's IDDA African Regional Symposium on Food Fermentation Technology held in Dakar, Senegal, from 13 to 16 December 1993, UNIDO's linkage with ARCT will be strengthened and promoted. ARCT will be identifying important African technical focal points for UNIDO-promoted global networks in the following areas: (1) traditional food fermentation technologies; (2) lactic acid fermentation technology; (3) cassava processing technology, a subject that encompasses not only food industries but also animal feed industries; (4) mushroom biotechnologies and bioconversion technologies for sustainable industrial development; and (5) marine biotechnologies. With the development of global linkages, African countries will then be able to enjoy increased opportunities for interregional cooperation and development within the functioning of these global networks.

An important point to note is that the December UNIDO IDDA Symposium in Dakar was held in cooperation with the African Agency for Biotechnology. The African Agency was formulated by UNDP in 1991 with headquarters in Algiers. The coming together of African scientists and specialists at UNIDO's Symposium will strengthen scientific expertise and extend linkages within the African Agency.

Discussed at the Networking Session of the symposium was a proposed UNIDO delivery mechanism for biotechnology transfer to, and cooperation among, countries in the African region that is to take place systematically, initially through consolidation of resources and the activities of ARCT, the African Agency for Biotechnology, and the African Regional Training Programme at the University of Zimbabwe. This UNIDO delivery mechanism can be envisaged in its operation as the completion and linking of a still incomplete system of train tracks between centres of activity on which will run the engine of biotechnology transfer as a multi-purpose tool for African industrial development.

In addition to these networking activities, UNIDO is actively developing a project for the building of databases identifying indigenous biotechnology expertise in African countries. The goal is to catalogue and link activities appropriate for commercialization and to build on the experiences of successful biotechnology transfer in other developing countries, in particular in developing countries in South-East Asia. The project is intended to enable the transfer of these African biotechnology activities to small-scale African enterprises and industries. Several international donor agencies have expressed interest in working with UNIDO to ensure the sustainable operation of this proposed Network for the Commercialization of African Biotechnologies.

UN and other organizations' news

Biodiversity treaty to come into force for 1994

The UN Convention on Biological Diversity came into force on 29 December 1993 following its ratification by 30 countries. The first meeting of the parties to the Convention will follow during 1994.

The treaty is designed to stem the loss of the world's estimated 10 million plant and animal species and thus its genetic resources. Article 1 of the Convention

states its objectives as "... the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources".

The Convention's intergovernmental committee met in Geneva in October 1992 to discuss national strategies for reversing the loss of biodiversity. A technical working group also examined how to decide which projects should be given priority and which international funding body should operate the Convention's finances. (Source: *European Chemical News*, 18 October 1993)

Why the Global Environment Facility needs reform

From the outset, biodiversity conservation was one of the Global Environment Facility's (GEF) four principal areas of concern. Forty per cent of the fund is designated for biodiversity, an amount that far surpasses the relatively small investments that have been made in this field over the past few decades. The GEF biodiversity projects range in cost from under $3 million in Cuba and Mauritius to $30 million investments under discussion for Mexico and Brazil.

Today, three years after its creation, the GEF is up for re-evaluation. It has immense potential to effect change, but major reforms are needed if it is to have real impact on protecting global biodiversity. At present, the GEF is a loose aggregate of different activities, a set of projects that are really a variety of experiments, some piggy-backed on existing World Bank or UNDP-financed projects, and others that stand alone. It is hoped that following the pilot phase, the GEF will work closely with the Biodiversity Convention adopted in Rio de Janeiro, ensuring a more strategic and prioritized approach.

Since its creation, the GEF has assembled a portfolio of some 44 biodiversity projects with a total value of approximately $300 million. Because of the large scale of many of these projects, the immediate threat lies in allocating millions of dollars to fledgling natural resource management sectors, which in many developing countries are unable to use the money effectively. Part of the problem lies in the GEF's current operating style: restricting its funding to large-scale projects channelled only through central governments. This is poorly suited to biodiversity conservation.

To be successful, biodiversity conservation requires a flexible approach, with short project cycles and smaller initial investments, supported by longer-term funding to ensure continuity. The GEF Small Grants Programme for Non-Governmental Organizations (NGOs) - initiated and managed by UNDP - is a good first step, but represents only 2 to 3 per cent of the total GEF portfolio and has been slow to come on line.

Moreover, most of the expertise on biodiversity and much of the capacity to implement field projects rests outside the government sector. Under the circumstances, project review should be placed more in the hands of national or international NGOs with extensive experience in a country or project site. Finally, the GEF should work with governments and the NGO community to take a serious look at biodiversity conservation priorities.

Through the GEF, the international community has - at long last - the financial resources to conserve our poorly understood but globally critical natural resource base. The GEF is in a position to use the valuable lessons of its first three years to design a more effective structure for future biodiversity conservation efforts. (Source: *Choices*, 1993)

From IBPGR to IPGRI

Following the ratification of its Headquarters Agreement and the publication of the Agreement in the *Gazzetta Ufficiale* of the Republic of Italy, the International Plant Genetic Resources Institute (IPGRI) has started to function as an independent institution of the Consultative Group on International Agricultural Research and as the successor to the International Board for Plant Genetic Resources (IBPGR). To ensure an orderly transition, IBPGR will continue to operate alongside IPGRI until April/May 1994.

While IPGRI will operate as an independent international institution, its long association with the Food and Agriculture Organization of the United Nations on programme matters will be maintained under a Memorandum of Understanding on Programme Cooperation, signed on 21 September 1990.

IPGRI was established as a legal entity under international law more than two years ago under the terms of agreement signed by the Governments of China, Denmark, Italy, Kenya and Switzerland. The agreement has been signed by an additional 20 countries: Belgium, Bolivia, Cameroon, Chile, Cyprus, Egypt, Greece, Hungary, India, Iran, Jordan, Pakistan, Poland, Portugal, Romania, Russia, Senegal, Syria, Turkey and Uganda.

IPGRI has four major objectives. First, it will assist countries, particularly in the developing world, to assess and meet their needs for the conservation of plant genetic resources and to strengthen links to users of those resources. Second, it will build international collaboration in the conservation and use of plant genetic resources, mainly through the support of networks on both a crop and geographical basis. Third, it will work to develop and promote improved strategies and technologies for the conservation of plant genetic resources. Finally, the Institute will provide an information service to inform the world's genetic resources community of both practical and

scientific developments in the field. (Source: *African Diversity* No. 8, February 1994)

WHO International Laboratory for Biological Standards

The National Institute for Biological Standards and Control (NIBSC) is a WHO International Laboratory for Biological Standards. A major aspect of its work is the development and establishment of international standards for biological substances, an activity which is underpinned by appropriate research and development projects, e.g. on the design of novel bioassays.

The Institute also undertakes batch release testing of biologicals including vaccines and products derived from human blood on behalf of the UK Department of Health and WHO.

Services available from NIBSC include:

(i) Preparation of biological reference materials, including filling into ampoules to high reproductivity and exacting specifications;

(ii) Freeze drying;

(iii) Pilot studies to assess candidate reference materials;

(iv) Testing of biological products.

For further information please contact (in confidence): The Director, NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK. Tel.: (44) 707 654 753; Fax: (44) 707 646 854. (Source: *EBIS*, Vol. 3, No. 4 (1993))

UNESCO holds first meeting of International Committee on Bioethics, 15-16 September 1993

The United Nations Educational, Scientific and Cultural Organization has set itself an ambitious task: "the exploratory study of the conditions for the possible drafting of an international instrument for the human genome".

Its chosen instrument, an International Committee on Bioethics (ICB) of 47 eminent lawyers, scientists and others, met in Paris under the chairmanship of Mme. Noelle Lenoir, member of France's Constitutional Council, and author of the report which preceded the three laws now in debate in the French Parliament.

UNESCO Director-General Federico Mayor opened the first meeting with a speech emphasizing the need to maintain freedom of research and access to knowledge as well as to control abuses. Mme. Lenoir presented the report of the Scientific and Technical Orientation Group, which had worked since December 1992.

The report concludes that "the time has come to draft an international standard-setting instrument, based on ethical guidelines, concerning:

- The status of knowledge
- Protecting the human being
- Safeguarding the human species
- Educating, training and informing the public."

There was some dissent as to the ICB's ability to address the intercontinental complexities of patent laws and the rationale for focusing on human genome research when existing and simpler science and technology can be and is unethically exploited, but the Committee commands great expertise, among its own members or by invitation, and UNESCO may succeed in developing an instrument commanding respect, without stigmatizing the new techniques or knowledge. Details: George B. Kutukdjian, UNESCO, Place de Fontenoy, 7, 75007 Paris Cedex. Tel.: (33) 1 45 68 38 14; Fax: (33) 1 45 06 07 72. (Source: *EBIS*, Vol. 3, No. 4, 1993)

The Intermediary Biotechnology Service

The Intermediary Biotechnology Service (IBS) was established by an international group of donor agencies, to act as an independent advisory service on issues of biotechnology research management and policy formulation, and on socio-economic and technical issues. It advises bilateral and multilateral development agencies on biotechnology issues affecting developing countries. Primary clients include national policy-making bodies, national agricultural research institutes, and other research organizations, both public and private, in developing countries.

The establishment of the IBS resulted from a recommendation from the Biotechnology Task Force (BIOTASK) of the Consultative Group on International Agricultural Research (CGIAR). BIOTASK recommended that a demand-driven, problem-oriented advisory service should be established. This service should make available the expertise of advanced biotechnology institutes to the developing countries, many of whom are already making large investments in biotechnology research.

The IBS is headquartered at the International Service for National Agricultural Research (ISNAR), and supported through a grant from the Government of the Netherlands. The IBS, which became fully operational in March 1993, is guided by a Steering Committee composed of representatives from client countries, contributing donors and ISNAR.

Programmes and activities

The current programme of the IBS has three main functions:

- To assist national agricultural research systems in developing countries with biotechnology research programme management and policy formulation;

- To carry out country studies to identify priority problems amenable to solution through biotechnology;

- To identify international biotechnology expertise and enhance its availability to national programmes in developing countries.

One of the first activities of IBS has been a survey on international agricultural biotechnology programmes. The information collected through the survey has been entered into a computerized database. With the development of this database IBS hopes to facilitate developing countries to sort out options for the implementation of biotechnology research programmes and to support linkages between the international biotechnology programmes and IBS clients. Another important tool is formed by publications which analyse policy-related issues involved in biotechnology research. To date, IBS has published the following reports:

- Agricultural Biotechnology in Developing Countries: A Cross-Country Review;

- Intellectual Property Protection of Biotechnology: Implications and Options for Developing Countries;

- Biotechnology Priorities, Planning and Policies: A Framework for Decision-Making.

Publications planned for 1994 include:

- Biotechnology, Economics and Market Analysis of Tropical Beverage Crops;

- Animal Biotechnology Initiatives for Developing Countries.

The November meeting provided IBS with an opportunity to consult with scientists and policy makers from developing regions on the next phase of its activities, which will focus on activities at the regional and national level. As a first step in this direction, IBS will organize two seminars, in Asia and Africa, in 1994. The purpose of these meetings will be to open a dialogue between government departments, scientific institutions, end-users and special interest groups on issues such as priority setting, planning and policy-making for biotechnology research. The regional seminars will also be used to determine themes for further work and assess the needs for IBS activities at the country level.

Contact: Joel I. Cohen or John Komen, Intermediary Biotechnology Service/ISNAR, P.O. Box 93375, 2509 AJ The Hague, the Netherlands. Tel.: (31) 70-3496100; Fax: (31) 70 3819677. E-mail: JKOMEN@CGNET.COM(Internet). (Source: *Biotechnology and Development Monitor* No. 17, December 1993)

Call for common research programmes

The Working Group on Technology and Agrarian Development (TAD), recently established at the Wageningen Agricultural University, the Netherlands, studies the possibilities for a "tailor-made approach" to technology design in all areas of agriculture. It currently concentrates on plant improvement for war-raged communities in Africa, and on users' perspectives in biotechnology for farmers in developing and developed countries.

The TAD group aims to draw conclusions about ways of effective participation of farmers' organizations and/or other client groups in biotechnological research. The TAD group hopes to set up interdisciplinary research programmes with persons and institutes in a number of developing countries.

Therefore, it welcomes contacts with any researchers or research groups interested in one or more of the following themes:

- Innovative roles of farmers in generating, maintaining and further developing of biotechnologies;

- Farming practices and techniques that result from an articulation between global and local bodies of knowledge;

- The reconstruction and adaptation of biotechnologies to local situations;

- Crop improvements for refugees and resettled groups in societies recovering from war;

- Possibilities to introduce user perspectives into international agricultural research programmes;

- Redesigning biotechnologies to meet the needs of sustainable farming styles in developing and developed countries;

- Introduction of gender dimensions in the design of new biotechnologies.

These issues will need to be studied by teams of researchers from different disciplines. The TAD group

hopes to facilitate the necessary interdisciplinary partnerships, based on inputs from working groups in Wageningen Agricultural University and developing country institutions. Those interested in PhD projects linked to the above-described subjects are also encouraged to seek contact

Researchers and/or research groups interested in exploring possible links with the TAD group of Wageningen University are requested to briefly describe the kind of research they are interested in, how this existing programme fits into their working environment and whether they might be able to stay in the Netherlands.

Address: Wageningen Agricultural University, Working Group on Technology and Agrarian Development, Prof. Dr. Paul Richards/Dr. Guido Ruivenkamp, Nieuwe Kanaal 11, 6709 PA Wageningen, the Netherlands. Tel.: (+31)8370-85030/82776 Fax: (+31)8370-84759 (Source: *Biotechnology and Development Monitor* No. 17, December 1993)

NGO Network on PGR to embark on African survey

Formed in October 1992, the African NGO Network on Plant Genetic Resources (PGR) has representatives from Zimbabwe, Kenya, Ghana, Senegal, and also PGRC/E in Ethiopia. It is now embarking on a regional survey on NGO activity on PGR in Africa. The Rural Advancement Fund International (RAFI) has been closely linked to the Network in an advisory capacity since its inception. Its chair is Andrew Mushita of COMMUTECH in Zimbabwe. (Source: *African Diversity* No. 8, February 1994)

REDBIO

REDBIO is the Technical Cooperation Network on Plant Biotechnology for Latin America and the Caribbean. It started in 1990 as a result of an official request made to FAO by the Governments of Costa Rica, Colombia and Chile. The general objective of the network is "to accelerate the process of adaptation, generation, transfer and application of biotechnologies, to contribute to the solution of the problems of plant resources of the countries of the region". Priority will be assigned to biotechnologies that represent comparative advantages for the solution of specific problems such as micropropagation, conservation and characterization of germplasm, cell and tissue culture, diagnosis of pathogens and molecular genetics.

A Regional Coordinating Committee prepares and promotes the work plan and a Consulting Technical Council establishes the priorities and policies and promotes the procurement of resources. The Technical Secretariat is performed by the FAO Regional Office in Santiago, Chile. Focal points for promoting the establishment of national networks have been designated in 16 countries. REDBIO includes at present 256 laboratories and institutions, public and private, in 20 countries. It hopes in the near future to expand to the English-speaking Caribbean countries and to some countries and institutions of Africa and the Near East. (Source: *Boletín de Biotecnología*, Vol. 10, No. 1, December 1993)

Regional Programme of Biotechnology for Latin America and the Caribbean

This Programme started in 1987 under the sponsorship of the United Nations (UNDP/UNESCO/UNIDO). Its Regional Managing Committee, representing 13 countries, has met six times in various Latin American countries. The Programme intends to be a suitable framework for the gestation of integrated policies for development in biotechnology and for the elaboration and implementation of coliaborative actions aiming towards the solution of regional priority problems through products, processes and services.

The activities have been divided into two subprogrammes: those projects relating to R&D and to the preparation of manpower in basic sciences have been under the responsibility of UNESCO; on the other hand, those tasks related to detection and evaluation of technologies and their possible use for industrial purposes are under the responsibility of UNIDO.

So far, nine projects have been financed, four of them in basic research: development of laboratory diagnostic procedures for Chagas' disease and leishmaniasis; development of new diagnostic systems for plant viruses; development of new markers for diagnostic probes in malaria, diarrhoea agents and hepatitis; genetically improved varieties of sugar cane and maize resistant to insects. The other projects are: enzymatic degradation of industrial wastes; industrial production of penicillin-amidase to obtain 6-aminopenicillanic acid; obtainment of an enzyme to hydrolyse lactose in milk; development of potato plants resistant to some viruses; mass production of monoclonal antibodies.

Both subprogrammes have organized symposia, workshops and training courses in subjects related to the research projects.

A second phase of the Programme intends to continue supporting the infrastructure already established in the region, to strengthen the integration and cooperation among countries, and the training of personnel at all levels. In the next three years, 10 courses in advanced technologies and in the application of new technologies to production will be offered on a regional basis (Source: *Boletín de Biotecnología*, Vol. 10, No. 2, December 1993)

Social issues

Bioethics and law issues stand at the genetic frontier of biotechnology

The legal and ethical issues arising from biomedical advances in organ transplantation and genetic testing areas

were recently addressed in a symposium entitled "Law and Science at the Crossroads: Biomedical Technology, Ethics, Public Policy and the Law" (Boston, Massachusetts), sponsored by Suffolk University Law School in cooperation with the University of Massachusetts Medical Center.

As surgical and related biomedical technology continues to advance, the number of organs needed is increasing at a rate that outpaces that of supply. The two major issues that must be continuously addressed are rationing of these scarce resources and increasing donor-organ availability.

The two basic approaches to increase donor-organ availability are one in which more organs are donated and one in which alternative sources are developed.

Alternative sources provide the clearest potential for closing this gap. Biotechnology companies have been quick to respond here.

The need to prevent immunological rejection is the unifying theme of the biotechnological approach. Masking foreign antigens has become the common goal. Antigens are being masked by modifying cell surface antigens and their expression, as well as by tissue encapsulation and the *ex vivo* use of artificial organs.

Competition to develop these alternatives is strong given that (1) estimated total world-wide organ transplants will grow to at least 42,000 by the year 2000, and (2) the knowledge that this figure is only 10-25 per cent of the number of transplants that could be performed.

While ethical questions remain, the public seems likely to accept alternative organ sources. Unfortunately, the inherent difficulty in surmounting the high technological barriers means that clinical trials will not start until late in this decade and any commercial impact will begin only after the year 2000.

The major medical problem currently is that the ability to detect genetic conditions far exceeds medicine's power to ameliorate or cure these conditions. Diagnosis will significantly outpace therapy for the next two decades.

Identifying genetic diseases now is primarily limited to the determination of susceptibility. The currently obtainable information relates to the propensity to develop a disease and not to its actual expression. The severity of genetic disease can vary dramatically in different generations for reasons that are not well understood. Most important for the biotechnology industry, all current predictive tests are still at the research level.

The overriding concern relating to genetic testing is the right of individuals to protect the confidentiality of the results. This right to privacy directly conflicts with the need of the health insurance industry to accurately quantify risk factors. In fact, the industry position is that access to genetic testing results is as fundamental as access to medical information in classifying risk. In short, genetic testing information is necessary to run insurance companies as businesses.

However, the confidentiality of testing results may be a moot point. Most information considered confidential is actually readily available in our computerized society. Genetic testing results will be no different. As a result, the emphasis should be on how to deal with the inevitable disclosure of this sensitive information.

The concern voiced by the symposium participants was that a positive test result for a genetic disorder will become a pre-existing condition that is excluded from health insurance coverage. One participant reported that the overwhelming consensus in Congress is that pre-existing conditions, presumably including identified genetic defects, will be covered without discrimination at a community rate. Under these conditions, either employers or healthy individuals will subsidize the premiums of those who test positive for a genetic defect.

In the past five years, gene therapy has progressed rapidly and has become a readily accepted medical treatment. However, it probably will not become commonplace until well into the next decade. Even then, genetic diseases that are attributable to the expression of one, or maybe a few, mutant genes will be the only ones treatable. The vast majority of genetic diseases are due to multiple defects that interact in a complex manner with normal gene expression and behavioural responses.

However, advances in biotechnology have allowed the ready identification of both types of genetic defects. Hence, the ability to identify a propensity towards a genetic disease has far outstripped the knowledge of how this propensity will impact the affected individual.

Since genetic testing results are difficult to interpret, at best, and involve sensitive privacy issues, testing companies must respond appropriately to avoid public backlash. Confidentiality and privacy must be of paramount importance in order to prevent potential abuse of results. Genetic testing companies will need to be acutely responsive to these issues to achieve commercial success.

Finally, the ability of individuals to make informed decisions based on the often complex results of genetic testing is limited.

Doctors and genetic counsellors are an integral part of this evaluation process. Genetic testing companies will need to encourage individuals to have their testing results evaluated as part of an overall counselling plan. However, until confidentiality can be assured or the impact of disclosure negated, individuals will be more likely to have testing done in isolation, hoping to maintain privacy. (Extracted from *Genetic Engineering News*, February 1994)

Regulatory issues

Call for EC regulations to converge with US

A new report compares bio-regulations in the European Community with those prevailing in the United States - and calls for an EC system much closer to the US system. Issued by Dr. Dieter Brauer of Hoechst and Prof. Dieter Schlumberger of Bayer, the report calls the US system "pragmatic, scientifically appropriate, and thus highly competitive". This system has evolved from experience with micro-organisms and plants and assumes that there is "no evidence that a unique hazard potential exists either through the use of rDNA techniques or through the transfer of genes between unrelated organisms". In contrast, Brauer and Schlumberger note that regulations in Europe reflect the EC Commission's view that, without exception, rDNA techniques require oversight and control specifically designed for rDNA operations. They conclude that it is "almost unthinkable" that the existing EC regulatory system - based on the 1990 Directives on contained use and deliberate release of genetically modified organisms - can allow universities and industry to adapt to scientific progress sufficiently rapidly to be competitive. Details from: Senior Advisory Group Biotechnology, Av. E. Van Nieuwenhuyse 4, bte 1, B-1160 Brussels, Belgium or on (+32)2-676-72-86. Fax: (+32)676-72-88. (Source: *Biotechnology Bulletin*, October 1993)

The Bioindustry Association endorses recommendations of the Select Committee report on biotechnology regulation

The Bioindustry Association (BIA), the trade association for UK bioscience companies, welcomed the publication of a report from the House of Lords Select Committee on Science and Technology on the "Regulation of the United Kingdom Biotechnology Industry and Global Competitiveness".* In particular, the BIA is delighted that the Select Committee clearly focuses on the regulatory regime as a burden on industry's capacity to innovate and compete in world markets.

The BIA strongly endorses the Committee's recommendations that Government must press for amendment of EC Directives and "streamline" current UK regulatory practice to enable UK industry and academic researchers to compete more fairly at an international level. The report focuses on the unscientific basis and unnecessary bureaucracy of much of the UK regulation covering biotechnology activities and how this hampers the competitiveness of UK industry.

The BIA draws specific attention to certain of the Select Committee's conclusions contained in the report:

- The "new" biotechnology of genetic modification is an exciting and continually evolving set of applications which are already in everyday use and result in well-known medicinal products, vaccines and household goods.

- The benefits of biotechnology are already well proven and its products will yield enormous benefits to mankind.

- Early fears of scientists relating to genetic engineering have turned out to be unfounded. With a few exceptions, separate regulation of genetically altered organisms for industrial or research use is unnecessary and their release into the environment is not inherently dangerous.

The BIA joins with the Select Committee on Science and Technology in recommending that Government considers urgently a reform of the United Kingdom regulations and applies strong pressure for amendment of relevant EC Directives.

Commenting on the report, the BIA's Executive Director, Mr. Louis Da Gama, said: "We have been enormously impressed by the thoroughness and depth of the Select Committee's inquiry. They have consulted widely and taken evidence from all sides of the debate on the safety of genetic engineering. We hope that the Committee's clear statement that genetic engineering is inherently not dangerous will help to allay public concern. The BIA hopes the Select Committee will encourage Government to take a more flexible approach based on increased knowledge, relevant experience and technical progress rather than on the currently over-restrictive non-scientific regulatory regime."

Further information available from: Louis Da Gama, Executive Director, Bioindustry Association. Tel.: 071 957 4600. (Source: *Bioindustry Association Press Release*, 13 October 1993)

Report on ecological risks of transgenic crops in global markets

Scientists have already succeeded in producing engineered versions of most of the world's major food and fibre crops - including corn, rice, soybeans and cotton. Since 1987, the US Government has approved hundreds of applications for field tests of genetically engineered crops, with many crops now in their third or fourth year of testing.

The major players in the development of engineered crops are multinational chemical and pharmaceutical companies. Although the number of products at the brink of commercialization is growing, many important issues surrounding commercialization of transgenic crops are still unsettled. Among these are the impact of such products on

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* Seventh Report of the House of Lords Select Committee on Science and Technology, HL Paper 80, published 13 October 1983, HMSO, London.

the sufficiency and viability of world agriculture and the question of environmental risk.

Regarding the former, there is increasing concern about the destructive effects of food production systems on the resources on which an exploding world population will depend in the twenty-first century. Many see the solution to these problems in a fundamental reorientation of agriculture towards sustainable practices that will evolve from a systems-based approach to the perpetual problems of yield, pest control and soil conservation.

Another important question for the future is whether genetically engineered crops will aid or retard the global transition to a sustainable agriculture. This question deserves intense public debate, which ideally should take place before the technology is allowed to become commercialized.

Also at issue are the health and environmental risks entailed in the wide commercial use of transgenic crops. This point has received some discussion in government and industry circles, but often from a narrow perspective that has downplayed the serious nature of the risks.

Yet, analysing even this restricted set of issues is a formidable challenge. If the agricultural biotechnology industry fulfils the hopes of its promoters, it could be producing hundreds of kinds of transgenic vegetables, grains, fruits, trees, fibre crops and ornamentals by the turn of the century. These plants will be grown on huge acreage around the world. Most of these crops will contain combinations of genes and traits not possible in nature. Moreover, in many cases, the novel genes will be transferred via pollen from the crops to populations of wild relatives.

What environmental risks would large numbers of these different varieties of transgenic crops pose? Ecological risks depend, among other things, on the nature of the crop, the characteristics of the added gene and the agricultural locale.

A recently completed report offers a framework to begin to analyse the environmental risks. It also offers a practical, innovative approach for assessing some of the ecological risks that affect decision-making in a regulatory programme.

The report reaches four major conclusions:

Commercialization of transgenic crops poses serious environmental risks. The widespread commercialization of transgenic versions of the full spectrum of food and fibre crops poses serious environmental risks that can be considered in several broad categories. These include the possibilities that:

- Transgenic crops themselves will become weeds;
- Transgenic crops will serve as a conduit through which new genes move to wild plants, which could then become weeds;
- Plants engineered to contain virus genes will facilitate the creation of new viruses;
- Plants engineered to express potentially toxic substances like drugs and pesticides will present risks to other organisms that are not the intended targets of the new chemicals.

The risks are similar in some ways to those presented by the introduction of non-native organisms into new environments. Most non-native organisms die out quickly in new environments. But occasionally one will take hold and, in the absence of ecological controls, lead to extensive damage.

Commercialization of transgenic crops could threaten global centres of crop diversity. Crop genetic diversity is already diminishing at a stunning rate, as farmers around the world are persuaded to abandon the numerous landraces of the past in favour of a relatively few modern crop varieties. Expensive transgenic plants, which will generally have to create large markets to recoup research costs, will exacerbate that trend.

Two aspects of the risks of transgenic crops can be assessed and minimized through a scientifically sound regulatory system, i.e., that transgenic crops themselves will become weeds and that novel transgenes will be transferred into wild populations.

Other aspects of the risk of transgenic plants are difficult to evaluate. The long-term, cumulative risks to ecosystems of introducing large numbers of transgenes and transgenic plants are not well enough understood to allow their prediction except in the grossest sense. It is unlikely that ecosystem dynamics will be well enough understood any time in the near future to confidently predict this aspect of environmental impact.

The Union of Concerned Scientists (UCS) calls on the Federal Government to adopt strong measures to protect against the domestic and, to the extent possible, the global, environmental risks posed by genetically engineered crops. The UCS recommendations included the following specific actions:

- The United States should establish a strong federal programme to assess and minimize the risks of transgenic crops before they are commercialized.
- All transgenic crops should be evaluated for two aspects of ecological risk - weediness potential and gene flow - before they are approved for commercialization.

- The Federal Government should develop standard protocols to assess the risks of creating new viruses.

- All transgenic seeds that are exported from the United States should bear a label stating that approval of the seeds under US law carries no implication of safe use in other countries.

- The appropriate United Nations organization should develop international biosafety protocols, which are necessary to ensure that developing countries, especially those harbouring centres of crop genetic diversity, can protect against the risks of genetically engineered crops.

Perils Amidst the Promise: Ecological Risks of Transgenic Crops in a Global Market, prepared by Jane Rissler, Ph.D. and Margaret Mellon, J.D, Ph.D, at the Union of Concerned Scientists in Washington, D.C. (Extracted from *Genetic Engineering News*, 1 February 1994)

General

Diagnostic MABs available from ATCC hybridomas

The American Type Culture Collection (ATCC) has recently released two interesting hybridomas from its cell culture collection. HB-10452, described in US patent 5,168,063 and deposited by the Wisconsin Alumni Research Foundation, secretes a mouse IgG_{2a} monoclonal antibody specific for enteropathogenic *Escherichia coli* serotypes 0157:H7 and 026:H11. A strain of 0157:H7 was implicated in the recent outbreak of haemorrhagic colitis from tainted meat on the West Coast. HB-10494, deposited by Cytogen Corporation in connection with US patent 5,162,504, secretes a mouse IgG_1 monoclonal antibody to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients.

Both lines are among the many patent deposits available from the ATCC for research purposes. They can be ordered directly through the ATCC Sales Department.

Further information: ATCC Marketing (Tel.: (301) 881 2600; Fax: (301) 816 4367). (Source: *Australasian Biotechnology*, December 1993)

Growing collection of human brain cDNA clones

The American Type Culture Collection (ATCC) now has over 7,500 human cDNA brain clones identified by partial sequencing for use in gene identification, study of gene families and other applications. The clones are from the laboratories of James M. Sikela, University of Colorado Health Science Center, and J. Craig Venter, The Institute for Genomic Research. The materials are supplied as either slants of uncharacterized plasmids or as frozen uncharacterized bacteriophage lysates.

Clone descriptions are available through several electronic media formats: (1) the ATCC PC Diskette Catalogue section on Clones, Vectors, Libraries and Hosts (available for a fee from ATCC); (2) on Internet (dbEST and Gopher) (connect to the ATCC catalogue database by typing "gopher merlot.welch.jhu. edu"); and (3) via modem connection to ATCC's free on-line database (dial 1-800-647-4719, 881-4909 for local Maryland callers, pressing ENTER, and answering the "user name" and "password" prompts with the word [common] followed by ENTER.

Further information: ATCC Bioinformatics Department (Tel.: (301) 231 5586; Fax: (301) 7770 1541). (Source: *Australasian Biotechnology*, December 1993)

Plant genes: must firms pay to play?

A barley gene imported free from Ethiopia protects the $160 million US barley crop from the yellow dwarf virus, but a potentially bigger blight now looms on the economic health of barley and other crops: a proposal requiring countries to pay royalties for plant genetic materials (PGM) used to create commercial varieties.

International agriculture officials have struggled for a decade as to whether Western countries should pay royalties on PGMs, which germ-plasm banks have provided for free, but developing countries have stepped up demands for such royalties in the wake of the Biodiversity Treaty, which mandates better protection - and remuneration - for biological resources.

Concern is voiced that PGM royalties could curtail creation of new crop varieties. For example, seed companies often combine traits to create a cultivar. If each trait's country of origin were to charge a 5 per cent royalty, "you can get to the point where the sum is greater than the whole". (Source: *Science*, Vol. 261, 27 August 1993, p. 1107)

The development of AIDS in Asia

In the mid-1980s, when the AIDS epidemic was already well established in North America and Africa, it was still little known in Asia. Now the United Nations Development Programme estimates that before the turn of the century, most new cases of infection will be in Asia.

The Asian Development Bank held a conference in Manila in September 1993 to try to work out how the spread of AIDS will affect Asian economies.

Some places are more threatened than others. In Thailand, if semi-official projections of 500,000 cases of AIDS by the year 2000 are borne out, the disease will cause an economic crisis. This number of victims could cost the Thai economy $18 billion, a figure equivalent to 23 per cent of GDP in 1990, although the cost will be spread over many years. The burden is particularly severe

because AIDS affects people in what should be their most productive years.

After Thailand, the country most under threat may be India, which is thought at present to have 300,000-500,000 HIV cases. Also at risk is Myanmar (Burma). The official estimate of the number infected, just a few thousand, is almost certainly too low.

The Philippines had discovered just 408 cases of HIV infection by June 1993. Malaysia had fewer than 3,000 reported cases. South Korea came up with just 494 positive tests, which may even be accurate. By the end of 1991 South Korea had carried out mandatory HIV tests on 1.3 million prostitutes, and had discovered only 25 cases of infection. But no country should feel complacent. People are increasingly mobile and so is the virus.

In Thailand people have not started to die in large numbers from AIDS. The first case was not registered until 1984 and to date there have been only a little over 5,000 cases of AIDS or AIDS-related complex.

As a result, some Thais pretend AIDS is not a problem. Some even suggest that positive tests for HIV are being confused with those for malaria. Sadly not. The spread of infection into the general population is illustrated by the fact that one in every 100 pregnant Thais tested has the virus.

In Asia, as in America, AIDS is increasingly likely to be a disease of the poor. One of the sadder studies presented to the Development Bank's conference concerned the relationship between the amount a prostitute charges and the likelihood that she will be HIV-positive. In the brothels of Chiang Mai, in northern Thailand, women charging up to 50 baht ($2) had an infection rate of 70 per cent. Women charging 100 baht had an infection rate of 16 per cent. (Source: *The Economist*, 18 September 1993)

Finding drugs in the rain forests

It is often said that the myriad species found in tropical rain forests may harbour cures to many diseases that afflict the world, and that the development of drugs based on these species could help pay for the forests' conservation. In Costa Rica, this is no longer just a saying, as Ana Sittenfeld, director of biodiversity prospecting at Costa Rica's National Biodiversity Institute (INBio), explained at a recent conference on sustainable development in Manchester.

INBio has joined forces with US drugs giant Merck in an ambitious project to try to develop drugs from rain forest species. INBio has trained local people as "parataxonomists", working with professional taxonomists and curators to identify animal and plant species in Costa Rica's conservation zone. This area covers some 12,750 km^2 and may contain up to 4 per cent of the world's land-living species. Samples of species are fractionated and analysed by INBio's extraction laboratory - which Merck fitted out at a cost of $180,000 - and screened for biological activity at the University of Costa Rica.

As well as laboratory equipment, Merck has provided $1 million towards the project. In return for this, Merck will be able to use INBio's database to look for promising drug leads. Because this database groups chemicals according to which species they came from, families of plants or animals can be investigated. If one species contains a useful compound, another in the family may contain a more potent form.

But the deal does not end there. If Merck's research brings drugs to the market, INBio will receive royalties from their sales. This money will then be ploughed back into rain forest conservation. This is the first time that the source of such drugs has benefited from their development. In the past, such research has been entirely one-way: the drugs companies have taken the rain forest's natural resources and given little or nothing in return.

Sittenfeld acknowledges that it may be many years before the fruits of INBio's inventory reach the market. And however successful the project is, it will still not provide enough money to support the Costa Rican forests.

While Merck may have plumped for Costa Rica, Pfizer has decided to stick to its own backyard. The US drugs company is to collaborate with the New York Botanical Garden (NYBG) in a $2 million, three-year project to collect and study plants, as sources of potential new medicines.

Under the agreement, NYBG will collect and identify plant samples from around the United States. Pfizer scientists will test extracts from those plants for their potential as disease-fighting agents or as leads to such agents. The company will pay a royalty to NYBG if a plant extract proves to be of any commercial use.

The British drug discovery group, Xenova, has got together with the FMC corporation and Purdue University, Indiana, to exploit Purdue's collection of 1,500 plant species from around the world. Purdue University will supply extracts and detailed background data to the British company for screening. Xenova will have exclusive rights to all human and animal pharmaceutical lead compounds while FMC will retain rights to any agro-chemical ones. (Source: *Chemistry & Industry*, 4 and 18 October 1993)

Life on Earth: mapping conservation priorities

Over the last five years we have seen international attention and concern grow, culminating at the 1992 Earth Summit with the completion of Agenda 21 and a major international Convention on Biological Diversity signed by 165 nations. Many donor Governments are now moving to incorporate biodiversity conservation into their individual

foreign assistance programmes, and are supporting the Global Environment Facility (GEF), the multilateral fund managed by the United Nations Development Programme (UNDP) and the World Bank, with technical support from the United Nations Environment Programme (UNEP). Yet despite these new initiatives, biodiversity continues to be lost at an alarming rate. Shockingly, an estimated 40-44 million acres of tropical forests are believed to be disappearing annually, taking with them a plethora of life forms. At this rate, the majority of the remaining tropical forests will vanish by the end of the next century. The time is now for the international community to set immediate action plans and priorities to promote conservation, and to invest more in programmes that demonstrate the true value of biodiversity.

The value of biodiversity conservation has slowly begun to emerge from the shadows. Ironically, it got its biggest boost when the US Government refused to sign the Biodiversity Convention in Rio de Janeiro, a move that generated tremendous press coverage and forced many Governments around the world to look at biodiversity for the first time. (The Convention was later signed by the Clinton Administration.) Despite growing public attention, however, the sum total of biodiversity knowledge is small, both in terms of the total diversity of species on Earth and the current and potential value of its use.

Science has thus far described only about 1.4 million species of animals, plants and micro-organisms. However, estimates and projections made in the last few years indicate that total species diversity on Earth could be as much as 10 million, 30 million or perhaps 100 million or more. What is more, our ignorance of the ecological processes involving this multitude of organisms is even more profound.

The other glaring gap in our knowledge concerns the value of biodiversity. Placing value on the services provided by Earth's many species and ecosystems is difficult and, as a quantitative discipline, poorly developed at best. Functioning ecosystems buffer the world against possible climate changes and shifts, for example, in crop yields and sea levels. Most often, however, discussion of biodiversity value revolves around the potential for biotechnology innovations and new pharmaceuticals (possible cures for cancer and AIDS from the tropical rain forest), or a handful of products from tropical countries that are currently traded internationally (timber, rubber and Brazil nuts from the Amazon, rattan from South-East Asia, etc.). The real value of biodiversity, however, based on actual current use is far more extensive.

While we may not always be aware of it, we all rely on biodiversity in our daily activities. In the United States, 25 per cent of all pharmaceutical prescriptions contain active ingredients from plants, such as quinine, while some 3,000 antibiotics are derived from micro-organisms. Another important example is coffee. Not only a daily beverage for millions, coffee is also a major export crop for many Latin American and African countries. It is, however, also susceptible to diseases like fungal rusts. To maintain the genetic viability of coffee crops, it is important to conserve wild relatives in their places of origin, like the highlands of East Africa and the eastern rain forests of Madagascar, where more than 50 wild species of coffee grow.

Conservation in Madagascar therefore may be essential to the future of global coffee crops. Likewise, conservation of certain areas is critical to agriculture as we know it. Agriculture accounts for more than 30 per cent of the gross domestic product of low-income developing countries. The value of agricultural trade is in excess of US$ 3 trillion annually, yet much of our global civilization rests on the cultivation of only seven grasses - rice, wheat, barley, oats, sorghum, millet and maize. These require continued genetic input from wild relatives of cultivars to maintain their resistance to pests and disease. Natural diversity of the wild relatives of these seven grasses must be maintained and seed banks are not enough to do the job, as little evolution and adaptation can take place there. It is therefore crucial to protect areas where the wild relatives of these critically important species grow. Furthermore, an additional 20,000 species of grasses exist, the vast majority of which are not being used.

Biodiversity also has an enormous recreational use, and connected with this is the spiritual and psychological well-being of our own species. Many studies have shown the damaging effects on the human psyche from long-term isolation from nature in urban environments. Increasingly, people are looking to the natural world for escape, especially in industrialized nations.

Biodiversity is by no means evenly distributed over the planet. Tropical rain forests provide us with a clear example. Recent estimates indicate that more than half of the Earth's original humid tropical forest has disappeared and about one quarter of the remainder is degraded, with some countries and regions (Madagascar, the Philippines, the Atlantic forest region of eastern Brazil) already having lost *up to 95 per cent* of original forest cover. In areas like these, we have at best the remaining years of the decade in which to put in place appropriate conservation measures.

First, we have to set priorities that focus heavily on the Earth's richest and most diverse areas now at greatest risk. One way to pin-point these areas is through the widely-used "threatened hotspots" approach. First conceived by the leading biodiversity expert Norman Myers, it focuses mainly on endangered tropical rain forests because overall they are the richest of the terrestrial biomes.

To date, some 15 priority tropical rain forest regions have been identified as "threatened hotspots". Together, they occupy approximately 4 per cent of the planet's land surface and harbour at least 30 per cent of all terrestrial life forms - and a much higher percentage of those at risk. Although still at a very preliminary stage of analysis, the "hotspots" approach has been very useful in enabling

governments, communities, international donors and NGOs to better target their conservation investments. With the goal of helping governments quickly assess the range of life forms present in threatened rain-forest areas, Conservation International developed the Rapid Assessment Program, which fields experts to a given location in the tropics to conduct quick surveys of its biological resources.

Efforts are also under way to determine "hotspot" areas of other major ecosystems or biomes. These include wetlands - such as the Pantanal of western Brazil, the Okavango Delta in Botswana and the Sudd Swamp of the Sudan. Also being studied are deserts, such as the Sonoran Desert of Mexico and the south-western United States and a number of large lakes, including the Rift Valley lakes of East Africa.

Other priority areas include specific countries that have vast biodiversity riches, the so-called "megadiversity countries". Some 12 countries are home to 60-70 per cent of the planet's life forms - including freshwater and marine life - and must therefore be strong candidates for international support. The richest of these countries are Brazil, Colombia, Indonesia, Madagascar, Mexico, Peru and Zaire.

Tropical forest areas that have remained pristine and largely intact - just the opposite of the "hotspot areas" - must also be prioritized for conservation efforts. These wilderness areas have great importance as storehouses of biodiversity, as major watershed areas, and are often the last places where indigenous peoples have any hope of maintaining a semblance of their traditional lifestyles. Moreover, they are likely to assume increasing recreational, aesthetic and spiritual value on our ever more crowded planet. These areas include the southern portions of the three Guianas, southern Venezuela and adjacent parts of northern and western Amazonia in Brazil, Colombia, Peru, Bolivia and Ecuador, the Zaire Basin and most of New Guinea.

Every country's biodiversity is important to its own viability and to the world at large, and must be conserved. What is emphasized is the need to recognize that certain parts of the planet have much higher concentrations of biodiversity than others, and that frequently these areas happen to be the ones at greatest risk. These places require a large share of our global investment in biodiversity conversation, perhaps even a share roughly proportionate to the diversity that they possess. Strategies for their conservation could include the establishment of parks and protected areas, major natural resource policy reform on the national, regional and local level, public awareness-building focusing on endangered species, and the development of markets for natural products - such as foods and oils that are harvested sustainably. Given the critical economic situation of many tropical countries, an emphasis on economically-based conservation alternatives is essential.

Protecting the world's biodiversity is an environmental challenge that cannot be addressed through technological innovation alone. Over the past several years the international community has made significant financial resources available to safeguard this poorly understood yet fundamentally important natural resource base. The opportunity to effect major change clearly exists but requires leadership, greater understanding of biodiversity and the will to set priorities and to take action.

While awareness is being built and funding generated, the challenge now is to translate these steps into concrete actions on the ground - on the front lines of the battle to protect the Earth's diverse and vital life forms. To succeed, these actions must be participatory in nature. They must be supportive of the economic needs and aspirations of local people, and build on their capacities to promote long-term conservation.

This article was adapted from "The GEF and Biodiversity Conservation: Lessons to Date and Recommendations for Future Action". Copies may be ordered from Conservation International, Legislative Programs, 1015 18th Street, NW, Suite 1000, Washington, DC 20036. (Source: *Choices*, 1993)

Time to bank on gene profiles

A data bank of DNA taken from people convicted of serious crimes should be set up, says the UK's Royal Commission on Criminal Justice in its report published in July 1993. The Commission also wants the police to have the power to take DNA samples from all suspects.

The Commission argues that DNA profiling is now so powerful a diagnostic technique and so helpful in establishing guilt or innocence, "we believe that it is proper and desirable to allow the police to take non-intimate samples ... without consent from all those arrested for serious criminal offences". It wants saliva reclassified as a non-intimate sample.

DNA from convicted criminals - in the form of profiles or samples - would be kept by the police in a data bank. The Commission says the data bank would "enable DNA evidence found at the scene of later offences to be compared with the DNA data of those who had previous convictions ... It would also enable unsolved earlier offences where DNA evidence had been found but not linked with the offender to be cleared up".

The Commission also wants more extensive collection of DNA samples for statistical work. This second data bank would be maintained by an independent organization, with safeguards designed to prevent police and prosecution from being able to identify who a sample came from. The Commission says the Government should legislate to enable these data banks to be established.

The civil liberties organization, Liberty, says any national DNA database must have adequate safeguards.

The Commission also makes a series of about 40 recommendations designed to improve the quality of forensic evidence. It wants a new Forensic Science Advisory Council to be set up to monitor the performance of all forensic laboratories, including the Home Office's Forensic Science Service and the Metropolitan Police's forensic laboratory.

The Council would help to lay down a code of ethics which would ensure that scientists appearing for the prosecution disclose all the forensic evidence in a case. It would also oversee arrangements for training forensic scientists.

The report also calls for pre-trial meetings between forensic scientists. "It is our belief that, in cases involving scientific evidence, the pre-trial phase should be used to sort out and define as many scientific issues as possible and to consider ... the best means of resolving [for example, by further scientific tests] any matters that may be disputed."

The Commission backs moves by the Home Office and the Metropolitan Police to make their laboratories available to the defence as well as the prosecution (except when they are already working for the prosecution). (Source: *New Scientist*, 10 July 1993)

Down to Earth: practical applications of ecological economics

The third meeting of the International Society for Ecological Economics is to be held from 24 to 28 October 1994 in San José, Costa Rica. Organized by the International Society for Ecological Economics, the Universidad Nacional - Costa Rica, and the Interamerican Institute for Cooperation on Agriculture, the meeting will address the growing global consensus that sustainability is the appropriate long-term social goal. The challenge is to devise practical methods to achieve sustainability at local, regional and global scales. The focus of the third biennial conference of the International Society for Ecological Economics will be to link theory with practice to produce practical applications of ecological economics in order to achieve sustainability. The conference will foster the broad transdisciplinary synthesis that is necessary to provide these solutions. The following topics are of particular relevance:

- Energy issues
- Population issues
- Equity issues: equitable distribution of resources (racial, gender, ethnic equity)
- Defence conversion and worker retraining
- Institutional restructuring for sustainability
- Employment impacts of sustainability
- Practical resource accounting
- Trade and environmental issues
- Envisioning alternative futures
- Ecological economics at community level
- Ecological economic literacy: increasing public awareness
- Applied ecological economic modelling
- Ecological economics of biodiversity
- Uses of economic incentives and disincentives
- Ecosystem restoration and conservation
- Ecotourism
- Clean technologies
- The role of the media

Further information is available from: III International Conference of Ecological Economics, P.O. Box 555, 3000 Heredia, Costa Rica. Tel.: (506) 60-1600; Fax: (506) 37-6868.

B. COUNTRY NEWS

Australia

Australia takes tough line on "HIV plant"

A potent chemical extracted from a common Western Australian shrub shows promise as a treatment against HIV - at least in the test tube. American researchers at the National Cancer Institute in Bethesda, Maryland, have isolated an alkaloid from the tough woody plant which halts replication of HIV, though they have yet to test its safety in animals, let alone humans. The NCI has applied for a patent on the chemical, which it calls conocurvone.

Richard Spjut, a botanist with the US Department of Agriculture, first collected specimens of the plant, which belongs to the genus *Conospermum*, in 1981.

One particular chemical isolated from the extract, a naphthoquinone trimer, prevented HIV from replicating and from killing a type of human immune cell. Dwight Kaufman, deputy director of the division of cancer treatment at the NCI, which oversees the screening programme, calls conocurvone "extraordinarily exciting".

Chemists are not yet sure how conocurvone inhibits HIV, but they believe that it works in a different way from the AIDS drug AZT (zidovudine) and its relatives ddI and ddC.

Of more than 7,000 plants screened by the NCI so far, *Conospermum* is the fourth found to have potential against HIV, along with plants from Samoa, Sarawak and Cameroon.

The chemists have also discovered that they can synthesize conocurvone from precursors found in the plant. Theoretically, as chemists have already synthesized these precursors, a pharmaceuticals company could produce conocurvone without having access to the plant

Conospermum is common in Western Australia, and both Australian and American officials are deeply concerned that people might start "poaching" it, endangering the supply even before researchers have determined whether conocurvone has any real value as a drug. A representative of the Western Australian Government warns that the active component is highly toxic to people.

A consortium of scientists from Western Australia is working with NCI to screen for conocurvone in other species of *Conospermum* and in plants growing under different conditions.

Australia has strong views on what the US should be allowed to do with a discovery from a plant that grows only in Western Australia. Inspired by the Biodiversity Treaty, government officials from Western Australia are aggressively asserting their right to benefit from the discovery. To that end, they are negotiating a unique collaborative agreement with their American counterparts which would ensure that they are included in virtually all future research on the chemical and the plant. (Extracted from *New Scientist*, 3 July 1993)

Brazil

Industrial-scale production of diagnostic enzymes begins

Brazil has begun producing enzymes for diagnosing diseases through genetic-engineering on an industrial scale, offering a 50 per cent reduction in the end price compared to that of similar products currently being imported.

The enzymes include Taq Polymerase, the principle component used for diagnosis by PCR (polymerase chain reaction).

The enzymes are being manufactured in the Molecular Biology and Infectious Disease Diagnosis Laboratory of the Oswaldo Cruz Foundation (Fiocruz). Wim Degrave, of Fiocruz, explains: "The plan is to meet the national demand for enzymes used for research and diagnosis until the Brazilian biotechnology industries achieve the capacity to absorb that technology".

Using a 5.5 litre fermentation tank, Fiocruz has succeeded in producing up to 100,000 units of Taq Polymerase annually. This is sufficient to make 20,000 diagnoses by PCR, meeting the entire national demand, at a cost ranging from $0.50 to $1.00 per unit. The income accrued by Fiocruz is being reinvested in research on the diagnosis of tropical diseases.

Another area to be explored by Fiocruz is the production of enzymes that are an alternative to Taq Polymerase, already being applied in other countries for diagnosis by PCR. Examples of these are TTH, produced by the bacteria *Thermus thermophilus*; Vent, synthesized by the *Thermococcus litoralis*; and Deep Vent, obtained from the bacteria *Irococcus furiosus*, discovered in volcanic larva in the ocean subsoil. It is the only one resistant to temperatures higher than 120° C.

Fiocruz is also producing 500,000 units per year of the enzyme BSPR-I, isolated from the bacteria *Bacillus sphericus*, and used for genetic identification in paternity tests. The product, unprecedented in the country, is being sold to private laboratories at $13 per lot of 1,000 units. In other countries, the enzyme used in paternity tests is HAE-III, isolated from the bacteria *Haemophilus aegyptius*.

Also manufactured are 2 million units annually of the enzyme ECO-R-I, applied for cutting off DNA in molecular biology research. It has the potential for being used industrially to identify *Tripanosoma cruzi*, which causes Chagas' disease.

Another enzyme, BCE-243, synthesized by Fiocruz itself for use in research, is being evaluated at the Joinville Biotechnology Center, which plans to produce it on a large scale to supply world genetic banks. This is an enzyme with high value added, costing $200 per lot of 1,000 units on the international market. (Source: *Gazeta Mercantil*, 6 April 1993)

Bio-Rio Science-Industry Park

This is the Brazilian answer to the challenge of science-industry integration. Federal University of Rio, Oswaldo Cruz Foundation and business corporations operate side-by-side in a modern complex integrating human and material resources for industrial research and development, within the area of biotechnology. The park started its operations in May 1989, and is managed by the Bio-Rio Foundation, an independent non-governmental, non-profit organization. Bio-Rio's main purposes are: integration of fundamental and applied research efforts; technology development and transfer; assistance to new biotechnology enterprises; industrialization of biotechnological products, with priority for health, agriculture energy and the environment; development of integrated programmes of human resources; international cooperation for the effective mastering of knowledge and technology transfer; promotion of technology-based joint venture; development of new markets and new products; generation of new investment opportunities. The facilities include: a Centralized Administrative Support Unit, Specialized Services Unit, Industrial Lots, and Technology Incubator. (Source: *Boletin de Biotecnologicia*, Vol. 10, No. 2, December 1993)

Oswaldo Cruz Foundation

FIOCRUZ, the Brazilian Oswaldo Cruz Foundation, fulfils its objectives and activities with 11 institutions as follows: Oswaldo Cruz Institute conducts research in biomedicine and postgraduate programmes, important biotechnology activities, applied to health, including some aspects of genetic engineering, and publishes the journal *Memorias do Instituto Oswaldo Cruz*; the School of Public

Health provides training at the postgraduate level: the Research Center René Rachou located in the State of Minas Gerais, conducts research in control, diagnostic, therapeutics, epidemiology and immunology of tropical diseases and in natural products: the Research Center Aggeu Magalhães, in the State of Penambuco, specializes in the study of local diseases of the north-east region; the Research Center Gonçalo Moniz, in Bahía, study local diseases including their molecular biology and immunological aspects; the Institute of Technology of Immunobiological Products (Biomanguinhos) is the main centre for the production of vaccines and laboratory re-agents of South America; the Institute of Technology of Pharmaceutical Products (Farmanguinhos); the National Institute for Quality Control in Health; the Institute Fernandes Figueira with responsibilities in researching, teaching and direct assisting health for women, children and adolescents; the House of Oswaldo Cruz, a museum of history of health and medical sciences; and, finally, the Polytechnic School of Health Joaquim Venancio, training good quality technicians.

FIOCRUZ produces vaccines for yellow fever, measles meningitis A and C, typhoid fever and poliomyelitis, for the country's needs. Other vaccines are in the research stage: dengue fever, schistosomiasis, fascioliasis. FIOCRUZ also prepare kits for the serological diagnosis of hepatitis, rotavirus, adenovirus, Chagas' disease (recombinant antigen), HIV-1, rabies, measles, leishmaniasis, toxoplasmosis, rubella, and leptospirosis. Some other items produced mainly for research purposes are: restriction enzymes, recombinant proteins, synthetic oligonucleotides, genomic DNA from *Mycobacterium*, monoclonal antibodies, insect pheromones, bioinsecticides and others. (Source: *Boletin de Biotecnologicia*, Vol. 10, No. 2, December 1993.

Canada

Agriculture Canada - Research Clonal Genebank

In 1989, Agriculture Canada established the Canadian Clonal Genebank (CCG) at the Smithfield Experimental Farm, Trenton, Ontario. The mandate of the Genebank is to protect and preserve the genetic diversity of Canadian fruit crops and their wild relatives for our future generations.

The germplasm preserved at the CCG constitutes a reservoir of genes and genetic combinations that plant breeders and researchers can use as a source of new traits to respond to environmental changes and new biological challenges. The collection is important internationally, as well as nationally. There are over 2,500 accessions or clones stored at CCG.

Whenever possible, the CCG will fill requests for plant material. Germplasm is distributed for research and breeding purposes only. For more information, contact Ms. Margie Luffman, Curator at CCG, 613-3923527. (Source: *The AgBiotech Bulletin*, Vol. 1, No. 2, March/April 1993)

Agriculture Canada - Regulatory

A new committee has been established to provide Agriculture Canada with recommendations regarding the registration of microbial products that are regulated under the Fertilizers Act. This committee will also supply the Department with advice on registration policies and guidelines. Membership includes representatives from the Government, university and industry sectors. Regulatory officials from departments such as Health and Welfare and Agriculture Canada will also participate with non-voting status. The committee participants will work to expedite the registration process and provide a forum for constructive information exchange. For further information contact Ms. Margaret Kenny, Plant Industry Directorate, Agriculture Canada (613) 995-7900. (Source: *The AgBiotech Bulletin*, Vol. 1, No. 2, March/April 1993

China

Osaka Medical College confirms Chinese medicine effective for AIDS infection prevention

Researchers Jiang Yan from China and Professor Masuyo Nakai of the Department of Microbiology of Osaka Medical College, have confirmed that the root of the giant knotwood, a Chinese herb that has been used for gastro-enteric remedy and diuresis, effectively prevents the AIDS virus cell infection.

Research is still in the *in vitro* stage. Jiang and other researchers have found that the root of the giant knotwood has fewer side effects. Further research is being undertaken in developing this herb for AIDS treatment.

Jiang and other researchers noticed that the giant knotwood, which grows wild, has been used in China for the treatment of 'flu or pneumonia caused by virus or bacteria.

In their experiment, the extract obtained by brewing the plant's root was added to a mixture of AIDS infected lymphocytes and non-infected normal cells. Without the extract of the plant roots, the fusion of infected cell and non-infected normal cells occurred, and the enlargement and eventual destruction of cells occurred after 24 hours.

With the addition of the plant root extract, the enlargement of cells rarely occurred and the destruction of cells by the AIDS virus was suppressed.

The lymphocytes infected by the AIDS virus were soaked in the solution of the plant root extract, then incubated for six days.

The AIDS virus soaked in the extract of the plant did not break out from lymphocytes. This observation was confirmed through an electron microscope. (Source: *Nihon Keizai Shinbun*, 10 April 1993)

Chinese medicine said to induce cancer cell self-destruction

At the 1993 International Symposium on Chinese Herbs, a research paper on the induction of apotosis of virus-infected cells and cancer cells was presented. The induction was caused by Tsumura's product, the *Tsumura thorowax root* (TJ-9). Apotosis is a concept relating to cell death.

Apotosis is interpreted as the self-destruction of cells. When cells become useless or abnormal, they become self-destructive and die. This destruction is programmed by pre-determined factors. During apotosis, typically DNA cleavage and an increase in cytoplasmic and nucleus concentration occur.

The research paper on apotosis induction of virus-infected cells was presented by Professor Hidechika Okada of the Biomolecular Department, Molecular Medicine Research Institute, School of Medicine, Nagoya City University. When Bicalane and Bicalene, the main chemical compositions of Tsumura's thorowax root, were added to the culture broth of the Human T cell, leukemia Virus-I (HTLV-I) and AIDS virus (HIV) infected cells, there was a cytotoxic phenomenon due to DNA cleavage. When added to the culture broth of a mixture of HIV-infected and non-infected cells, Bicalane and Bicalene had a tendency to suppress the spread of infection from an HIV virus to a non-infected cell.

Bicalane and Bicalene have been known to activate the inhibition of reverse transcriptase of HIV virus cells. This recent research data indicates that Bicalane and Bicalene may selectively obstruct or destruct HIV infected cells

Apotosis of cancer cell research was presented by Professor Masamichi Kumashiro, Department of Pathology Laboratory I, Kurume Medical University School of Medicine. Clinical data have been reported on "TJ-9", which prevents the progression of hepato-cirrhosis to liver cancer. Latest research seems to basically support the data. Tsumura and Co. has applied for patents of TJ-9 and its related components for use as an anti-viral and anti-malignant tumour drug. (Source: *Kagaku Kogyo Nippo*, 13 April 1993, p. 9)

Tests for cancer

It is difficult to untangle the causes of liver cancer in a typical clinic in Shanghai. In the areas around the clinic, people live crowded lives and as a result many are exposed to the same pathogens and toxins - including aflatoxin and hepatitis B, both known causes of liver cancer. In order to determine the role of aflatoxin, which is a toxic by-product of mould on peanuts and corn, in these cancers molecular methods are used.

Armed with some of these new tools, a new form of therapy is to be tried in a Chinese clinic. First, two molecular-based bioassays are to be used to find vulnerable individuals: a urine test that looks for a "point" mutation (a single base pair change) in DNA, along with a blood test that looks for a change in haemoglobin. Surveys using these methods, together with blood tests for hepatitis B, have already yielded startling results. Near Shanghai, the chances of getting liver cancer are doubled for people exposed only to aflatoxin, and they are increased five-fold for those exposed to hepatitis B. But for those exposed to both, the risks jump to an incredible 60 times the risk for non-exposed people.

To break this lethal synergy, the focus is on aflatoxin and high-risk individuals are to be given a protective drug called oltipraz - formerly used as an anti-schistosomiasis medication. It stimulates metabolic enzymes that interact with aflatoxin compounds and divert them from the liver, where they damage DNA. Already, by giving oltipraz to aflatoxin-exposed rats, tests have shown that it blocks carcinogenesis.

This attack on aflatoxin could be used in other areas, including, for example, South Africa and the Gambia, where close living makes it difficult to sort out the effects of aflatoxin and hepatitis.

The general clinical research centre at the University of Michigan, Ann Arbor, has developed a breath test that looks at the rate at which people use a member of the cytochrome P450 enzyme family to metabolize erythromycin. In this test, a carbon-14 labelled form of erythromycin is injected, and the patient is asked to blow bubbles into a liquid that captures the exhaled carbon dioxide. Fast metabolizers, indicated by the ratio of labelled carbon atoms, are at increased risk for developing aflatoxin-related cancer. (Source: *Science*, Vol. 259, 5 March 1993, p. 1,395)

High-quality snake venoms

Dongtai Industrial & Trading Company, headquartered in Guangzhou City, Southern China, is of a special interest in biological products, particularly biotoxins. Their major item for export is high-quality lyophilized snake venoms produced by their own snake farm, one of the largest in China. With a steady monthly output and rich stock, they are in a position to supply the following:

Major items:

Lyophilized Venom of Naja naja atra (Chinese Cobra);
Lyophilized Venom of Agkistrodon Halys (Halys Snake);

Lyophilized Venom of Agkistrodon Actutus (Hundred-pace)

Other items:

Scorpion Venom
Bee Venom
Biliflavine
Bilirubin
Haney
Venom Defibrase
Thrombin
Heparin Sodium
Heparin Calcium
Salted Pig Casing for Sausages
Pig Bile Acid
Phytic Acid
Citric Acid
Arsenic

Further information is available on request from Wei Keqin, Import and Export Division, Dongtai Industrial and Trading Company, Room 538, Parkview Square, 960 Jie Fang Bei Road, Guangzhou, China. Tel.: 86-20-6665666, Ext. 538, 539. Fax.: 86-20-6673050. (Source: *News Release*, 18 August 1993)

Colombia

Biotechnology Institute of the National University of Colombia

This Institute was established in December 1987 as a multidisciplinary group from different schools of the University of Colombia to meet national industrial needs. The Institute serves as a centre for the coordination of research carried out at its own and at various other laboratories. The central laboratory is a complex which occupies 15,000 sq.ft. where nine laboratories, a Biotechnology Orientation and Reference Centre, a conference room and various offices are integrated. At present a staff of 120 work at the Institute, including professors, researchers, technicians and students. It also includes laboratories at the schools of Engineering, Medicine, Sciences and Agronomy. It gives support to graduate and undergraduate academic programmes, particularly in chemical engineering, industrial design, biology, chemistry, pharmacy, microbiology and biochemical engineering.

The three main research areas are:

1. **Agricultural biotechnology**, with projects in molecular biology of viruses, in rhizobiology and in bio-insecticides;

2. **Diagnostic systems**, with projects in molecular biology of *Escherichia coli* and *Vibrio comma*, in ecotoxicology, in the development of probes for the detection of foot and mouth disease virus and the production of a recombinant vaccine, in the preparation of kits for plant viruses detection, and in the identification of species-specific ribosomal sequences;

3. **Biochemical engineering and enzyme technology** with projects for the production of 6-amino-penicilanic acid (6-APA), and in molecular biology of *Clostridium acetobutylicum* for the production of butanol and the hydrolysis of lactose in milk.

(Source: *Boletin de Biotecnologicia*, Vol. 10, No. 2, December 1993)

Cuba

Genetic Engineering and Biotechnology Center

The Genetic Engineering and Biotechnology Center of Havana, Cuba, started operations in 1986 in a large and comfortable new building.

Its main industrial products are: a hepatitis recombinant vaccine (Heberbiovac HB); recombinant streptokinase (Herberkinasa); a skin healing cream with Epidermis Growing Factor (Hebermin); recombinant Alpha Interferon 2B (Heberon Alfa R); natural Alpha Interferon (Heberon Alfa N); Transference Factor (Hebertrans); and some other recombinant proteins plus about 26 types of monoclonal antibodies. It also produces diagnostic kits for AIDS (HIV 1 and 2), for hepatitis B and C and for toxoplasmosis. Some other biological products from the Center are: restriction enzymes, DNA molecular weight markers, oligonucleotides and synthetic genes. Two vaccines against human meningitis and colibacilosis of cattle and pigs are meanwhile produced by genetic engineering.

For industrial biotechnological purposes the Center produces some enzymes such as: prokimosine, lactase, sucrose-invertase, renin, beta-galactosidase and alpha-amilase.

In the agricultural area, research is being done on plant and cell micropropagation to obtain new varieties resistant to insects, fungi and virus diseases. Some work is performed in the area of nitrogen fixation and in the production of transgenic animals.

The Center also performs analyses on the purification and characterization of proteins by mass spectrometry, determination of tri-dimensional structure of proteins by X-Ray diffraction and nuclear magnetic resonance; also, electron microscopy and immunomicroscopy are included in the facilities of the Center. (Source: *Boletin de Biotecnologicia*, Vol. 10, No. 2, December 1993)

European Community

Biotech companies shun Europe

European biotechnology legislation is deterring US companies from investing in the EC, while European-based companies are eyeing up opportunities in the US rather than in Europe. Dr. Kenneth Baker, director of biotechnology, science and technology at Monsanto, told a select committee of the UK House of Lords that he and other European-based executives of US companies have an uphill task arguing against the perceived negative climate for biotechnology.

Baker's concern is echoed by a poll conducted by the Brussels-based Senior Advisory Group on Biotechnology, which indicates that EC-based firms may abandon Europe and place future investments in the US.

According to SAGB, the US will receive the lion's share of investment by European-based biotechnology users. More than 45 per cent of companies polled said that the US would be the target for investment in manufacturing facilities, while only 35 per cent said that they would locate within the EC. Japan and the Far East look set to attract substantial investment from about one in five EC-based companies.

SAGB officials blame the tangled bureaucracy of EC biotechnological regulations for the exodus and are calling on the European Commission to speed up the promised review programme. (Source: *European Chemical News*, 17 May 1993)

European Commission aims to spotlight bio-opportunities

The Commission for the European Communities (CEC) is implementing several priority actions designed to improve the competitiveness of European industrial sectors which benefit from biotechnology. One of these actions, which aims at the establishment of a Community network for training and research, has been under way since 1982.

- It began with the *Biomolecular Engineering Programme* (BEP: 1982-86, 15m ECU).
- Next came the *Biotechnology Action Programme* (BAP: 1985-89, 75m ECU).
- Then came the *Biotechnology Research for Innovation, Development and Growth in Europe* programme (BRIDGE: 1990-93, 100m ECU), currently in progress.
- The Community action will continue in the BIOTECH programme (164m ECU), which will run from 1993-97.

The extensive R&D activities carried out under these various programmes have given rise to a pool of highly trained personnel and to significant advances in scientific discovery in the field of biotechnology.

To encourage the transfer of the resulting knowledge and technology to industry, the CEC has set up a parallel programme known as VALUE.

The VALUE programme is aimed at small and medium-sized enterprises (SMEs), which could benefit from using new technologies - but do not have the resources to keep track of the latest technological developments. It can also provide funding to aid companies in assessing and commercially exploiting new technologies. BioResearch Ireland (BRI) has been co-contracted by the CEC (with support from VALUE) to set up and operate a system to disseminate information on biotechnology R&D results and highlight technology opportunities. (Extracted from *Biotechnology Bulletin*, June 1993)

The Plant Industrial Platform

A Plant Industrial Platform (PIP) was established at a meeting of some 20 companies involved in plant biotechnology, in Lyon on 20 May 1992. Seventeen companies have joined the PIP since:

- Zaadunie B.V. (The Netherlands);
- ICI Seeds (United Kingdom)
- VanderHave Research (The Netherlands)
- Florigene B.V. (The Netherlands)
- ICI Seeds/SES (Belgium)
- Keygene N.V. (The Netherlands)
- R.Z. Biofleur (The Netherlands)
- Royal Sluis B.V. (The Netherlands)
- L.V.M.H. Recherche (France)
- Nickerson Biocem Ltd. (United Kingdom)
- Monsanto Europe SA (Belgium)
- Biocem SA (France)
- Rhone Pôulene Agrochemie (France)
- Peto Italiana S.R.L. (Italy)
- Planta Pflanzengenetik Biotech. GmbH (Germany)

- RAGT SA (France)

- Pioneer Hi-Bred S.A.R.L. (France)

The platform will provide means and opportunities for the exchange of results, materials and techniques between industry members and the academic laboratories involved in the two BRIDGE T-projects. New plant T-projects, to be initiated in the future, will be able to profit directly from PIP.

The platform will concentrate on scientific liaison and will not become involved in political or regulatory affairs, nor produce position papers. These areas are already dealt with by existing organizations such as the Green Industry Biotechnology Platform.

Membership is open to any European company and is not limited to the EC.

Information presented in T-project newsletters will be made available and industrial contributions will be stimulated.

PIP members will be able to receive written documentation, lists of publications from T-projects, the available basic information on the projects and gain opportunities to contact responsible scientists.

The existence of the platform will not affect contractual rights of companies or scientists already participating as contractors in the EC T-projects. (Source: *The Genetic Engineer and Biotechnologist*, Vol. 13, No. 2, 1993)

Defending biotech

Spurred on by the need to protect research investments, the European Union's internal market council has agreed to support the patenting of biotechnological inventions - except where they include modified versions of human genes. The draft directive will now proceed to the European Parliament.

Not all countries were in favour of the proposal, however. Spain, Denmark and Luxembourg voted against it on ethical grounds. The Spanish and Danish parliaments are currently in the throes of national debate over biotechnology ethics.

The Italian delegation also opposed the directive in its original form, arguing that some experiments involving the human genome are an affront to human dignity. However, Italy eventually agreed to support a directive banning the patenting of modified versions of human genes.

The directive, part of the White Paper on the completion of the single market, is due to come into force by 1996. "The issue needed to be addressed as a matter of urgency, otherwise we could carry on watching a brain drain to countries where patents benefit from a better protection", said the Belgian chairman of the council Robert Urbain. (Source: *Chemistry & Industry*, 3 January 1994)

Virus resistant transgenic plants

The Commission of the European Communities (CEC) BRIDGE programme is currently supporting a project titled "Molecular biology of the cell-to-cell movement of plant viruses in relation to plasmodesmatal structures". Studies on the viral proteins that allow plant viruses to enter healthy cells during the infection cycle ("cell-to-cell movement proteins") have shed new light on both the viral mechanisms involved and on the plant structures which are exploited. Cell-to-cell movement proteins are normally expressed from viral DNA during the infection cycle and facilitate spread of newly synthesized viral particles into healthy cells. It has been proposed that transgenic plants which express mutated forms of the cell-to-cell movement protein may interfere with cell-to-cell spread. This approach could therefore be exploited to develop virus resistant transgenic plants.

The genes encoding cell-to-cell movement proteins of several viruses, including the alfalfa mosaic virus (AlMV) and the cauliflower mosaic virus (CaMV), have been cloned and expressed in yeast, baculovirus and *E. coli*. Mutants of the AlMV and CaMV genes have been constructed and transformed into plants where they express modified forms of the cell-to-cell movement proteins. The researchers are currently attempting to verify that this strategy is successful in conferring plant resistance to the corresponding virus. Similar experiments are also in progress with cucumber mosaic virus, tobacco mosaic virus and beet necrotic yellow vein virus. If this approach proves successful the researchers are interested in transferring the technology to other commercially important crops, for example, cucumber, melon, zucchini, etc. Further research funding is needed to facilitate this. Contact is sought from companies involved in plant breeding or plant protection who may be interested in commercial exploitation of this technology under a joint venture or licensing agreement. European companies interested in commercially exploiting these results are eligible to apply to the CEC-VALUE programme for financial assistance towards feasibility studies and product development.

For further information on this research and on the CEC-VALUE programme please contact, Dr. Constant Gitzinger, Commission of the European Communities (DG XIII). Tel.: (+352) 4301 33887/33519, Fax: (+352) 4301 34129. (Source: *Irish Biotech News*, January 1994)

Innovation in agro-biotechnology

The EU SAST Programme has produced a series of reports on the prospects for biotechnology innovation in the agricultural area. The reports deal with the areas listed

below and for each they examine the benefits which can be gained from application of biotechnology and the major economic, social and regulatory factors which effect its application. The reports make recommendations as to the orientations of the EU's R&D and other programmes based on their findings.

The set of project reports comprises six case studies on:

- Lower levels of fertilizers and pesticides in agricultural crop production;
- Plant breeding technology;
- Animal production;
- Non-food uses of European agricultural production;
- Agro-industrial development in Portugal;
- Characterization and measurement of quality in agro-industrial production.

SAST Report orders (quoting ref. CD NA 14716 to 21) should be addressed to the official sales outlet for EC publications established in most countries, or to the Office for Official Publications of the European Communities, 2 rue Mercier, L-2985, Luxembourg. Fax: 352 407915.

Further information can be obtained from the SAST Unit at the Commission of the European Communities, Directorate-General for Science, Research and Development, 200 rue de la Loi, B-1049 Brussels. Source: *Irish Biotech News*, January 1994)

S&T Cooperation with central and eastern Europe (COPERNICUS)

The EC Commission is due to launch a Call for Proposals for COPERNICUS at the end of January, 1994. The programme has a budget of 55m ECU.

The Sectors covered will include Biotechnology and AgroFood Industries, as well as Information Technology, Communication Technologies, Telematics and Language Engineering, Manufacturing, Production, Processing and Materials, Measurements and Testing.

All firms (including SMEs), research institutes and universities, will be eligible. Projects must have one partner in the EC and two partners in two different central and eastern European countries: Albania, Bulgaria, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, Slovenia and the Czech Republic and additionally Armenia, Azerbaijan, Belarus, Georgia, Kazalshoton, Krygroton, Moldova, Russia, Yajikintan, Turkmenistan, Ukraine and Uzbekistan.

Project should be of not more than 500,000 ECU, of three years minimum duration, and a minimum of 75 per cent should be spent in Eastern Europe.

Priority themes are rehabilitation of economics; quality control, reliability, safety, recyclability, application of results. (Source: *Irish Biotech News*, January 1994)

Egypt

Egypt's cornerstone for agricultural biotechnology: AGERI

Dr. Magdy Madkour, Director
Agricultural Genetic Engineering Research Institute (AGERI)
Giza, Egypt.

The Agricultural Genetic Engineering Research Institute (AGERI), represents a vehicle within Egypt's agricultural arena for the transfer and application of biotechnology. The original establishment of AGERI in 1989 was the result of a commitment of expertise in agricultural biotechnology. The foundation of AGERI is the second phase of the national goal for excellence in genetic engineering and biotechnology. AGERI now aims for the adoption and application of the most recent technologies available world-wide to existing problems in Egyptian agriculture.

AGERI is located within the Agricultural Research Center (ARC) site which facilitates an interface with ARC's ongoing research programme and provides a focal point for biology and genetic engineering for crop applications in Egypt. The premises contain a total net area of 116m^2 consisting of 14 modern laboratories, a BioComputing and Networks Unit, a central facility, a preparation/washing facility and a supply repository. In addition, a 140m^2 "Conviron" controlled environment chamber has been recently installed to host the transgenic plant material for acclimatization; a 307m^2 fibreglass greenhouse has been added; and a new containment facility, based upon a University of Arizona design, is now being constructed adding 412m^2 of state-of-the-art space which will allow the safe handling of materials in experiments dealing with the degree of gene expression in transgenic plants.

The scientists work on several projects representing a spectrum of increasingly complex scientific challenges ranging from tissue culture technology to the transfer of genes controlling biotic and abiotic stress, growth, maturity and quality.

The senior scientists at AGERI have made a significant impact on the quality of education made available to young researchers at AGERI through their affiliation and teaching responsibilities in Egyptian universities. This academic linkage has brought about numerous regional and national training courses organized by AGERI staff including courses on:

- RFLP/RAPDs/PCR - For Crop Improvement
- PCR/ELISA - For Virus Diagnostics
- Modern Methods in Microbial Molecular Biology

With such interaction between AGERI, various agriculture-research centres and Egyptian universities, the education of students and the public alike has begun.

The effective handling of information is necessary for research and innovation. For this purpose, AGERI has employed the BioComputing and Networks Unit with the goal of supporting research activities and providing researchers with fast and efficient access to data and information located within Egypt and abroad. Services provided by this unit are:

- World-wide networking and communication capabilities;
- Maintaining a software library;
- In-house repair and maintenance of personal computers and laboratory equipment.

Further information may be obtained from AGERI, 9 Gamaa Street, Giza 12619, Egypt. Tel.: (202) 727831; Fax: (202) 629519; E-mail: nagel@egfrcuvx.bitnet (Source: *BioLink*, Vol. 1, No. 3)

France

France placates farmers with plant fuel plan

The French Government is pushing ahead with an expensive plan to step up biofuel production after pressure from the country's farming lobby.

The Government will spend £40 million on a three-year biofuel experiment in which 100,000 hectares of land will be used to produce 175,000 cubic metres of rape methyl ester (RME). The Government lifted fuel taxes from biofuels in 1992 and cities such as Dijon, Grenoble and Mulhouse are already experimenting with RME in their buses.

Under Europe's revised Common Agricultural Policy, French farmers must set aside 1.5 million hectares of land from food production. French farmers have mounted a strong lobby for the promotion of plant fuel. The Government's long-term objective is to use 700,000 hectares for fuel.

Biofuels would be produced most efficiently in the Paris basin which is far from being the most economically depressed farming region in France.

Growing, transporting and manufacturing RME would burn fossil fuels equivalent to 53 per cent of the RME's energy value, and would cost the State the equivalent of its total tax revenue on diesel. (Source: *New Scientist*, 27 February 1993)

Germany

Germany eases genetic legislation

Germany's federal cabinet has drafted an amendment to the 1990 genetic engineering framework law, easing restrictions for companies and research institutes working in the field. The changes have been welcomed by the VCI, which says they will improve competitiveness of German companies.

The amendment is scheduled to take effect at the beginning of 1994. It foresees a streamlining of declaration and approval procedures for genetic experiments in safety stages I and II and does away with the mandatory hearing for industrial projects in safety stage I, which by definition of the law are assumed to be "no risk" projects. Almost all genetic engineering projects in Germany fall into stages I or II. Stage III applies to "moderate risk" gene-splicing projects, stage IV to "high risk".

A hearing will be mandated for projects in stage II (low risk) only if they are done in plants subject to federal emissions control regulations. For most projects in stage I, only a declaration procedure - and no formal approval procedure as under the original legislation - will be required.

No changes have been made in the regulations calling for public participation in approval procedures for projects involving the release of genetically manipulated plants or micro-organisms. (Source: *European Chemical News*, 7 June 1993)

Field trials with genetically modified plants planned

The Institute of Plant Genetics and Cultivated Plant Research (IPK) in Gatersleben intends to apply for permission to perform field trials with genetically modified cultivated plants. Preparations have progressed to the point where tests can begin no later than 1994.

One of the products concerned is a potato with a genetically modified metabolism, which is to be tested for stress resistance under environmental change. The scientists also intend planting a relation of the bean with improved seed protein quality, approaching that of animal protein. The tests are intended to establish how the plants behave under day/night alternation and under natural weather influences. So far there have been only three trials involving the release of genetically modified plants in Germany. (Source: *Die Welt*, 14 June 1993)

Max Planck Institute develops photosensitive biofilm

Long before the first algae appeared, halobacteria "invented" photosynthesis and began to convert sunlight into energy. While man today tries to mimic this trick with solar cells made from silicon, halobacteria convert energy using a protein in their cell membrane known as bacteriorhodopsin. It is a highly complex molecule, the discovery of which earned German scientists Hartmut Michel, Johann Deisenhofer and Robert Huber a Nobel prize in 1988.

Bacteriorhodopsin has now advanced to become a high-tech material and is about to replace the silver salts used in films. This molecule can not only be used to make films that can be exposed and erased again at will, but it could also store more data in a smaller space than all the materials used to date. The Max Planck Institute considers the biofilm extremely significant - it could be as important for optical image and information processing as the development of the light-sensitive silver coating once was for conventional photography.

Initial trials showed the bacterial protein to be extremely durable: it does not age, it does not decay, and it always reacts in the same way to incident light, namely by changing colour from violet to yellow, up to about 100 times a second. If the molecular light converter is placed between two glass plates, it forms what may well be a record-breaking biofilm capable of storing up to 5,000 lines a millimeter. (Source: *Die Welt*, 27 May 1993)

India

Scientists seeking words from the wise, tap knowledge of India's women farmers

Scientists at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have discovered that women farmers cultivating India's marginal lands can teach them a thing or two. Women do a major share of the production, processing and preservation of food grains and cash crops in the semi-arid tropics. They learned from experience and also preserve a wide selection of seeds of locally-adapted crop varieties, which form the basis of food security in their resource-poor, risk-prone regions.

ICRISAT decided to tap this practical knowledge to bridge the gap between theory and practice. Scientists searching for the wonder seeds of new pest- and disease-resistant lines to augment genetic diversity and increase yields are sometimes out of phase with the realities of farming. When crop varieties in the controlled environments of research stations are not subject to the biotic and abiotic stresses encountered in farmers' fields, plant breeders and farmers work at cross purposes. What plant breeders consider useless is acceptable to farmers, while varieties breeders recommend are of little use to farmers. ICRISAT turned to the increasingly respected process of participatory research to ask women farmers what research was worthwhile.

Taking pigeon pea (*Cajanus cajan*) as a test case, entomologists asked a group of women farmers in a harsh area of Andhra Pradesh to compare four advanced pest-resistance lines - ICPL 84060, ICPL 87088, ICPL 87089 and ICPL 332 - with locally-available varieties under their own field conditions and management.

The results were surprising. The women rated three of the ICRISAT pigeon pea varieties - ICPL 84060, ICPL 87088 and ICPL 87089 - superior to the landraces. The fourth variety, ICPL 332, scored higher than landraces on several agronomic characteristics, but the women rejected it because it had a bitter taste. ICPL 84060 scored best overall. It yielded 0.94 tons per hectare, and the pod-borer caused only 5 per cent pod damage.

Despite the advantages of ICPL 84060 the farmers wanted to grow their landraces along with the other improved varieties they had tested. They believed that pest attack was lower when they grew a mosaic of varieties than when they grew a single one. They also wanted to take advantage of the higher yields by selling the seeds of the landrace in the local market and keeping for themselves the better tasting ICRISAT varieties, not yet available to other farmers.

The programme was unique in several ways. It involved only women farmers in a male-dominated society. The evaluation of varieties went well beyond conventional parameters for measuring yield and pest resistance. Most important, the women farmers offered suggestions on what future research and development might be most fruitful. And, according to Michel P. Pimbert, principal entomologist at the Legumes Programme of ICRISAT, scientists listened to the advice. (Extracted from *Ceres-cope*, January-February 1994)

Contributions of NBRI to floriculture

The National Botanical Research Institute (NBRI), Lucknow, over the years, has evolved more than 181 new cultivars of ornamental plants belonging to 15 genera through the processes of natural selection, hybridization, induced mutation through gamma-irradiation, photoperiodic control, tissue culture techniques, etc. The Institute has also worked on the commercialization of some of the popular flowers, such as Gladiolus spikes and Chrysanthemum. Several cultivars of Amaranths have also been evolved for their strikingly beautiful foliage. The rich germplasm of ornamental plants maintained by the Institute has the largest collection of Chrysanthemum and Bougainvillea, numbering about 450 and 250, respectively. Chrysanthemum cultivars like "Birbal Sahni", "Green Sleeve" and "Chandi" have won wide acclaim. The Institute occupies the position of a national reference centre for Chrysanthemum and Bougainvillea for possessing the largest number of authentic varieties of these ornamentals.

Besides, it has the richest collection of Amaranths (about 400 cvs.) in the country, which have been taken up for cultivation all over the country. The Institute has the choicest collection of exotic and Indian bred roses (500 cvs.) and has developed about 19 new mutant cultivars evolved by irradiating budwood of 35 cultivars of roses with 3, 4, 5 krad of gamma-rays using ^{60}Co as radioactive gamma-irradiation source. Eleven mutants of these have already been released as new cultivars for commercialization. (Extracted from *CSIR News*, 15 April 1993)

Italy

Italy adopts EC legislation on genetic engineering

With the passing of Legislative Decrees Nos. 91 and 92, published in the ordinary supplement to the Government GAZETTE No. 78 of 3 April 1993, the EC directives Nos. 90/219 and 90/220, regarding the confined employment of Genetically Modified Micro-organisms (GMMOs) and the deliberate release of Genetically Modified Organisms (GMOs), have become applicable in Italy. As far as the application of these decrees is concerned, it has been announced that the Ministry of Health will be the main authority, working in collaboration with the other departments involved. On the whole, these two legislative decrees appear to have been designed to meet the need of those working in this sector, not only in industry but also in universities and research centres, and to make them subject to the same kind of regulations governing genetic engineering as those currently in force in most of the other European countries.

Restrictions on the use of genetically modified micro-organisms

The decree makes provision for various classifications when giving notification of equipment and operations. Depending on the estimated level of risk posed by the GMMOs employed, these are classified in two groups:

- Group I, which includes those micro-organisms that pose no risk;
- Group II, which includes micro-organisms posing possible risks. Depending on the scale and the type of use, operations are divided into:
  - Type A, for use on a small scale, for research and development, and for teaching purposes.
  - Type B, for use on a large scale, and/or for industrial or commercial purposes.

Notification has to be given to the Ministry of Health of all plants and laboratories in which GMMOs are used, within 60 days of the coming into force of the decree. Within the same time limit, separate and detailed notification must also be given of all Type B operations (that is, large-scale or industrial operations) employing GMMOs falling under Group I, while notification must be given of all operations, whether Type A or Type B, which employ GMMOs falling under Group II.

After the expiration of the 60-day time-limit specified, any organization that intends to make confined use of GMMO's for the first time is obliged to proceed as follows: if Type A operations using Group I-type GMMOs are being performed, a statement detailing these activities must be prepared and held available for inspection by the authorities, in the case of Type B operations, notification has to be given. Notification must be given of all operations that involve the use of Group-II-type GMMOs, and in addition, operations of this type may not be initiated until explicit authorization has been received from the authorities. Notifications should include:

- Administrative and logistical details;
- Details of the microbiological system;
- Details of the installation and its purpose;
- An evaluation of the risks posed to man and to the environment.

Deliberate release of GMOs into the environment (plants, animals, micro-organisms, fungi)

The decree makes provision for various procedures, depending on the type of use made of genetically modified organisms:

- Experimental releases and field tests for research purposes;
- Release onto the market of products containing, or comprising, GMOs.

In all cases, notification must be given to the authorities of the intention to proceed with the release of GMOs into the environment. This notification must be accompanied by an evaluation of the estimated risks posed to the environment. No releases may be effected until approval has been received from the Ministry of Health. If this approval has not been received within 60 days of the notification being given, then permission should be considered as having been denied.

For commercial releases, it will be necessary to follow the authorization procedure laid down by the EC.

The following information must be given in notifications of experimental releases:

- Administrative and logistical details;
- Information regarding the GMOs;

- Information regarding the release and its purpose;

- Interaction with the environment and security measures.

In addition, notifications of commercial releases must also include the following:

- An evaluation of the risks posed to man and to the environment;

- Information on marketing and use.

The two decrees prescribe that notification must be given of all laboratories, and certain operations, that make use of Genetically Modified (Micro) Organisms to:

- The Ministry of Health, Department of Public Hygiene;

- Division V - Biotechnology Notification Unit, 60 Via della Sierra Nevada - Rome 00144, Tel.: 06-59944209; Fax: 06-59944249.

In collaboration with the Higher Institute of Health, forms have been prepared containing detailed instructions on how to set out the notification for the confined use of GMMOs.

Forms for the notification of the deliberate release of GMOs are annexed to the relative legislative decree.

Further detailed information and forms containing the lines to be followed when setting out the notifications are available at the Association's (Biotechnology Association) offices. (Source: *Assobiotec Lettera*, May 1993)

Japan

Clinical testing of liver drug jointly developed with China

It has been announced that Taisho Pharmaceutical Company and the Beijing Institute of Materia Medica of the Chinese Academy of Medical Science have jointly developed a drug called SY-640. SY-640 has already entered Phase II clinical tests as a hepatoprotectant. This is the first case of Japan-China joint drug development reaching the level of clinical tests. Taisho Pharmaceutical signed a joint research contract with China in 1984, and so far the research has consisted of extracting chemical compounds from natural substances such as herbal medicines and screening them at Taisho's Omiya laboratories. SY-640 is a chemically modified compound obtained from a herbal preparation of the bark of the Amur cork tree (*Phellodendron Amurense* Rupr.). Taisho Pharmaceutical owns the rights to this substance for the whole world except China.

It is believed that SY-640 acts as a hepatoprotectant by strengthening the cell membranes of hepatocytes, but the pharmacodynamics are yet to be clarified.

Hepatitis spread by hepatitis viruses is on the increase, and Taisho Pharmaceutical has strong hopes that if SY-640 confers hepatocytes with resistance to these viruses, it will develop into a major drug for treating patients afflicted by viral hepatitis.

Joint research between China and Japanese pharmaceutical companies with the goal of discovering new drugs from Chinese herbal medicines is increasing. However, the only other company that has a product developed to the level of clinical tests is Tsumura & Co., who is conducting preclinical studies on REN-1, a substance obtained from *Rehmannia glutinosa*, which is being tested as a drug to counteract lipemia.

In addition to the Beijing Institute of Materia Medica, Taisho Pharmaceutical signed a joint research contract with the Nishikawa Antimicrobial Industrial Laboratories in 1990. (Source: *Kagaku Kogyo Nippo*, 24 May 1993)

Science council urges DNA data bank expansion

The Japan Science Council recently compiled a report entitled "Urgency of expanding Japan's DNA data bank". The report points out the growing danger of criticism from Europe and the US that Japanese researchers are getting a free ride in terms of data. The report proposes the establishment of a Life Sciences Data Research Center at the National Institute of Genetics, a research organization shared by researchers throughout Japan, to integrate sophisticated R&D and data services. The report urges the expansion of the Japan DNA databank not only to fulfil Japan's responsibilities internationally, but also to support the growth of the life sciences and bioindustries domestically.

The Japan DNA databank (DDBJ) was established in 1984 under the auspices of the Related Research Office at the National Institute of Genetics. Together with GENEBANK from the US and EMBL Data Library from Europe, the DDBJ forms a three-member "International Shared DNA Database Organization" and must fulfil international duties on the world's DNA databank.

From an international perspective, Japan's contribution (based on number of data entries) is less than 8 per cent of the total and Japanese researchers use DDBJ much less frequently than the internationally shared DNA databases. Therefore, there is a strong likelihood that complaints about Japanese researchers getting a free ride will emerge from the USA and Europe. The committee fears that DDBJ will be removed from the international organization and Japanese researchers will be unable to use it.

Despite this situation, it is certain that the amount of DNA data requiring input will increase rapidly because of advances in the Human Genome Project and the Rice Genome Project, both of which are national projects. The committee believes that it will be necessary to acquire ¥900 million annually for operating and computer expenses and to increase the staff by ten within two years as a temporary measure if DDBJ is to meet its foreign and domestic demands.

In addition, the committee believes a life sciences data research centre built upon the DDBJ should be established to train personnel needed for the DNA databank and to provide efficient R&D services. (Source: *Nihon Kogyo Shimbun*, 19 May 1993)

MITI feasibility study on industrial applications of biomolecules

MITI has asked The Association for the Progress of New Chemistry (ASPRONC) to do a technical feasibility study on biomolecular engineering starting in FY93. In the field of biomolecular engineering, research has already made advances in protein engineering but this study will not be limited to proteins and will also include carbohydrates, lipids and nucleic acids. The study will look at basic technology to enable the design and synthesis of functional chemical substances based on clarification of their mechanisms for expressing activity. MITI will continue the study for about one year and plans to begin biomolecular engineering as a new national project in FY95.

Expectations are rising for the industrial application of biomolecules such as proteins as a new industry for the 21st century. In 1986 MITI established the Protein Engineering Research Institute (PERI) with an investment from the Japan Key Technology Center. The role of PERI is to clarify structure-function correlations of functional proteins such as enzymes that control bodily functions and to conduct R&D on the design and synthesis of proteins having rational functions.

PERI studies proteins, but there is a wide range of biological molecules other than proteins such as carbohydrates, lipids and nucleic acids. MITI is considering a new project that will involve clarifying the structures of all kinds of biological molecules, building a database, and artificially designing and synthesizing them.

While this study is underway, in order to begin the new national project in FY95, MITI will set up a preliminary group for establishing the new project that will include companies currently involved in PERI such as Mitsubishi Chemical and Toray and companies that want to participate in the new project. MITI will begin to study setting up an R&D structure, problems with funding, etc., and make a request for FY94.

Biomolecular engineering, which is considered third generation biotechnology, is a technology that is expected to have an impact on a wide range of disciplines such as chemistry (catalysts, highly functional materials), electronics (biosensors, biochips), medicine (anti-cancer drugs, physiologically active substances) and foods. (Source: *Kagaku Kogyo Nippo*, 25 March 1993)

Kenya

Sweet genes

A Kenyan scientist plans to introduce genetically engineered sweet potatoes to the farmers of her country. The chemicals giant Monsanto is training plant virologist Florence Wambugu to create virus-resistant transgenic strains of the crop. The idea is that Wambugu will return home to field test the plants, and train other African scientists in biotechnology.

Sweet potatoes are the world's sixth most important food crop, feeding 400 million people in Asia, Africa and Latin America. The plants conserve the soil, do not require herbicides or fertilizers and are drought-resistant, but viruses transmitted by aphids, particularly the feathery mottle virus, reduce the sweet potato crop yield in Africa by more than 50 per cent.

Wambugu and Robert Horsch, her colleague at Monsanto in St. Louis, have succeeded in transferring a gene from the protein of the feathery mottle virus into a sweet potato cell. They have now grown 200 of the virus-resistant plants. Wambugu is importing African strains to St. Louis so that she can create transgenic varieties adapted to the African environment. (Source: *New Scientist*, 27 February 1993)

The Netherlands

Dutch okay streamlined rules for GMOs

Dutch biotechnology researchers now have an effective one-stop shop for getting permits to conduct experiments with genetically modified organisms (GMOs), as officials at the Dutch Ministry of the Environment (MoE, The Hague) recently introduced a streamlined and harmonized permit-authorization process. "We now have one regulation implementing the European Community's (EC, Brussels) contained-use directive and deliberate-release directive", explains Piet van der Meer, the MoE's biotechnology legislation coordinator.

The new process takes much of the burden away from the local authorities. Previously, Dutch municipalities - of which there are about 700 - were responsible for authorizing permits under a law that was about 90 per cent harmonized with the EC's GMO regulations.

The local authorities still have much of their original power, but are not burdened with the technical problems associated with authorization. Industry now has a

harmonized and consistent interpretation of the rules. And environmentalists will get easier access to information by getting it from one source rather than 700 different ones. (Source: *Bio Technology*, Vol., 11 December 1993)

Russian Federation

Russian Federation seeks international advice on biotechnology regulation

Russia's Ministry of Science and Technology Policy has established a National Committee for Elaboration of Legislation for Work with Genetically Modified Organisms (NCLWG). This Committee is chaired by Professor K.G. Skryabin, Director of the Centre for Bioengineering of the National Academy of Sciences; and includes representatives of various interests, including the Ministries of Agriculture and of Ecology. They have been asked to draft a law by end of February 1994, for consideration by the new Parliament of the Russian Federation.

The International Centre for Genetic Engineering and Biotechnology, at Russia's request, organized an international meeting on 30 September - 1 October in Moscow at which Russian scientists and senior political figures exchanged information and discussed biotech safety and regulatory issues with a group of experts.

In a separate meeting, Professor K.M. Dynmayev, first Vice-Minister of Science and Technology Policy, stressed the importance of international cooperation in R&D and summarized the five main federal programmes in Life Sciences and Technologies:

- New methods of bio-engineering/biotechnology;
- Genetics: fundamental problems;
- Equipment and reagents, including a teaching programme;
- Human genome (with EC, US and HUGO links);
- Bio-diversity (linked to the decisions at the Rio Earth Summit, i.e. the Convention on Biological Diversity).

Referring to the group under Professor Skryabin, he emphasized Russia's wish to develop regulations in cooperation with the international community, compatible with international practice. Professor Dynmayev also mentioned the urgency of addressing public information aspects.

Debate has been wide-ranging, including the Church. Public reassurance was a recurrent theme in the sessions which followed.

Common aspects included an emphasis on the dynamic character of the development of biotechnology and the consequent need for regulations to be flexible and adaptable in scope and in detail. There was general recognition of the need to move towards a sectoral approach, and of the absence of scientific basis for treating rDNA organisms differently from other biological entities.

The consensus was for a product and risk-based approach, considering process only so far as relevant to risk assessment. The pros and cons of multiple laws and of a single law were debated. The Russians were clearly keen to learn from and avoid mistakes that had been made elsewhere.

Other contributions touched upon work on transgenic animals (so far, agricultural species); and upon the recent Russian law establishing property rights for animal and plant varieties (Russia is not a signatory of UPOV, the International Union for the Protection of Plant Varieties).

Extensive documentation was exchanged. The Centre for Bio-Engineering is an information centre for Co-Biotech, the Biotechnology Committee of the International Council of Scientific Unions; and has also been designated as a node of the UNIDO/ICGEB Biosafety Information Network and Advisory Service (BINAS). The UNIDO-led guidelines on field release have been translated into Russian. (Source: *EBIS*, Vol. 3, No. 4, 1993)

Western European and former Soviet scientists form links

Steps towards collaboration between scientists of Western Europe with those in the former Soviet Union got under way at the first general assembly of the Association for the Promotion of Cooperation of Scientists, held in Luxembourg. Created on the initiative of the Commission of the European Community, its main aim is to support scientific activities between scientists and research institutes of its members. Those involved in the inaugural event were the EC Commission, the 12 EC countries, and Austria, together with Armenia, Belarus, Russia, Ukraine and Uzbekistan. Membership is open to any country or to national non-profit organizations. Participants approved spending some $5 million to fund more than 50 projects - in physics, earth sciences and the environment, life sciences, economics and social sciences, and mathematics and information sciences - and adopted a provisional 1993 budget of about $23 million. (Source: *C&EN*, 5 July 1993)

Thailand

Thai-German biogas programme

Increased large-scale livestock farming, particularly of pigs, and the growth of agro-industry combined with

more stringent environmental regulation in Thailand are opening up new perspectives for biogas technology.

"Biogas technology is the only waste treatment process we know that allows even partial cost recovery", says Dr. Piyawat Boonlong of Chiang Mai University (CMU) and coordinator of CMU's part in the Thai-German Biogas Programme (TG-BP).

Bioenergy is not yet economical in itself. However, according to TG-BP estimates, biogas from their new plants, when converted to electricity, can reduce overall waste treatment costs in Thai piggeries by roughly one third. Water re-use and sale of fertilizer from the digested sludge could increase cost recovery to 40 per cent or more.

The programme is a joint undertaking between Chiang Mai University, the Department of Agricultural Extension and Gesellschaft für Technische Zusammenarbeit (GTZ), the German technical cooperation agency. Work began in 1988. The viability of its improved biogas technology has been demonstrated among smallholders in five northern provinces around Chiang Mai. The programme is now focusing on medium- and large-scale piggeries in the region.

A Modular Double Biogas System (MDBS) was developed by TG-BP to treat wastes. The core of the process is an Upflow Anaerobic Sludge Blanket (UASB) digester that rapidly processes the bulk of the less heavily polluted waste water from the piggery. UASB water retention time is five days or less.

The more heavily polluted fraction passes through a more conventional fixed dome or channel digester where retention times are up to 50 days. Slurry from the large digester is separated into solid fertilizer and further feedstock for the UASB. Both digesters produce biogas.

Seven of these installations now serve pig raisers in north and north-east Thailand. Early results are so promising that the programme wants to expand to west and central Thailand.

Investment costs for the system are high - about US$ 65,000 for a 500m^3 plant that could treat wastes from 1,800 pigs. Some farms raise 10 times that number and more. However, the electricity from biogas offers better cost recovery the bigger the system becomes.

A difficult problem is that not all farms can use all their biogas. And excess gas production is too small to be bottled. Ideally, producers should be able to sell their gas as electricity to the Electricity Generating Authority of Thailand (EGAT). But as Dr. Piyawat says, "At the moment, EGAT prefers to deal with only large producers such as sugar refineries".

However, everything may yet work out. Gas volumes could be large enough to receive proper attention. In any case, even partial gas use represents at least some cost recovery and a cleaner environment. (Extracted from "A Bigger Role for Biogas", *Biogas Forum*, 1993 III, No. 54, pp. 7-9)

United Kingdom

Calls mount for cut in UK biotech regulations

British industrialists have welcomes a call from the House of Lords Select Committee on Science and Technology for fundamental changes to UK biotechnology regulations. Zeneca, the BioIndustry Association and the CIA all urged the UK Government to heed the Lords' recommendations and cut the red tape they say is threatening to strangle the commercial development of the industry.

The Committee called existing regulations "excessively precautionary, obsolescent and unscientific".

Industrialists particularly welcomed the conclusion that genetically modified organism-derived products should be regulated according to the same criteria as other products, as they are in the US, and not on the basis of the process used. The Lords saw no case for the universal, generic labelling of GMO-derived foods or food constituents.

The Lords want drastic revision of the EC contained use directive so the size of an operation is no longer considered a component of risk. They also want the consent procedure simplified and speeded up so that so-called safe organisms only require notification and consents are given well within the 90-day maximum.

"The Byzantine structure of deliberate release regulation must be reformed so as to enable certain activities, as selected by a group of EC national experts, to be exempt from the present provisions", the Lords say. In the interim, they believe the current risk assessment questionnaire should be streamlined and all applications processed within 30 days. They also recommend that academic researchers should be exempt from application fees.

Meanwhile, the UK's Advisory Committee on Releases to the Environment has unveiled a plan to streamline GMO deliberate release applications. It will introduce a three-tiered evaluation system which will fast track those organisms that are thought to pose no risks and are well understood, while organisms thought to pose the most risk will get the most scrutiny. Organisms posing no risk but not so well characterized will be assessed as they are now. (Source, *European Chemical News*, 18 October 1993)

Crop centre for a greener revolution

The UK's leading centre for research on arable crops is throwing open its doors to agricultural scientists from developing countries in an attempt to spread new but

appropriate technologies to farmers in the Third World. The Institute of Arable Crops Research is offering 30 to 40 places each year to scientists who will learn about agricultural technologies and techniques that would benefit their countries.

Rothamsted International, a trust set up to administer the scheme, was launched in July 1993 when the Rothamsted Experimental Station in Hertfordshire celebrated its 150th anniversary.

So far, the trust has raised around £1.75 million, £45,000 of it from staff at Rothamsted. The remainder comes from charities, donations and industrial sponsors supporting fellowships.

Anderson, the head of the trust, says that the technology could help to increase yields of crops in a sustainable way, helping to feed burgeoning populations as land available for cultivation shrinks. "During the Green Revolution, many mistakes were made in transferring high-technology methods of agriculture into developing countries which could not sustain them practically or economically", says Anderson. The trust's approach will be to export only appropriate technology.

Anderson estimates that to keep pace with population growth, annual yields of cereals per hectare will have to increase from 2.7 to 5.7 tons by the year 2010. "We need to improve the rate of production and quality of the products with minimization of environmental impact", he says.

Rothamsted plans to teach visiting researchers a broad range of technologies - from rapid diagnosis of viral diseases and inoculation of roots with nitrogen-fixing bacteria to fertilizer management and the removal of heavy metals from soils. Genetic engineering techniques could be used to develop drought-resistant crops. "They would very much be generic technologies", says Anderson.

Besides running fellowships, the trust will work through joint programmes with institutes in developing countries, such as the International Institute of Tropical Agriculture in Nigeria and the International Crops Research Institute for the Semi-Arid Tropics in Andhra Pradesh, India. It will also operate closely with United Nations agencies, such as UNESCO, and hopes to forge links with associated British bodies such as the Natural Resources Institute of the Overseas Development Administration. (Source, *New Scientist*, 10 April 1993)

No ban on human genes in food

Consumers should be given enough information about genetically engineered foods to allow them to make informed choices about what they eat. But unless foods contain foreign genes they need not be labelled, even if they are produced with the help of genetic engineering. Sugar made from genetically modified beet, for example, would be exempt.

These recommendations were made in a report by the Committee on the Ethics of Genetic Modification and Food Use, set up to advise Britain's Ministry of Agriculture, Fisheries and Food. John Polkinghorne, president of Queen's College, Cambridge and the committee's chairman, says the group sees no need for an "absolute ban" on genetically engineered foods, including those that might in future contain genes of human origin.

The committee recommends that any food containing copies of human or animal genes should be labelled. Some organizations would want more information about foods than could fit on a label, and they would have to question manufacturers.

Committee member Peacocke says if genetically engineered foods "become very widespread, there may be some need for a central database" of the genetic engineering processes that manufacturers use for making particular foods. (Source, *New Scientist*, 25 September 1993)

United States of America

Long-term growth pegged for biotech

US sales of biotech products are expected to exceed $23 billion by 2004, growing at 15 per cent from 1994 sales of $6 billion, according to Consulting Resources (Lexington, MA). The firm estimates growth in human therapeutics at 14 per cent per year and expects sales to reach $16.5 billion in 2004; growth in diagnostics is pegged at 11 per cent per year, reaching $3.6 billion in 2004. Consulting Resources estimates sales of agricultural biotech products will grow to $1.5 billion in 2004, up from $130 million in 1994. (Source, *Chemical Week*, 26 January 1994)

UCSF awarded NIH gene therapy core centre

UC San Francisco was chosen to spearhead a major National Institutes of Health effort to cure genetic diseases through the establishment of a gene therapy core centre. The UCSF centre's mission is to develop new treatments and apply discoveries to treat cystic fibrosis, sickle-cell anaemia and other inherited genetic disorders by correcting genetic defects through gene therapy. It is one of three core centres to be established with support from the National Institutes of Diabetes and Digestive and Kidney Diseases. NIH funding for the Centre will be up to $2.25 million over five years. The Cystic Fibrosis Foundation is contributing additional support for at least three new CF research projects through the Centre. (Source, *Genetic Engineering News*, 1 November 1993)

Amendments to biotechnology regulations at USDA's APHIS

A final rule is now in effect amending USDA's biotechnology regulations. The Animal and Plant Health Inspection Service (APHIS) has added notification and

petition processes, as options in the existing framework for the introduction of certain genetically engineered plants. These changes are possible because of the abundance of scientific data gathered over the last six years from almost 400 field tests. Under the notification process, six crops - corn, potato, soybean, tomato, cotton and tobacco - may now be imported, moved interstate or field tested without first applying for an APHIS permit as long as certain eligible criteria and performance standards have been met. APHIS will still require information about the regulated article. Notification must reach APHIS at least 30 days before the crop may be field tested or imported into the country. Other crops may also be eligible for introduction under notification on a limited basis after a thorough case by case review.

The petition process allows anyone to submit, in writing, a request that a regulated plant should no longer be regulated. The petition must include detailed scientific information.

The biotechnology amendments were published in the March 31 Federal Register, Vol. 58, No. 60, pp. 17044-17059. For more information contact Ms. Sally Van Wert, Biotechnology Permit Unit, APHIS at (301) 436-7612. (Source, *The AgBiotech Bulletin*, Vol. 1, Issue 3, May/June 1993)

Biotechnology firms exempt from investment regulations

Emerging biotechnology companies and other R&D-intensive companies can invest their extra cash in stocks and bonds without being regulated like an investment company, according to a new Securities & Exchange Commission (SEC) policy. SEC is exempting biotechnology firms from the Investment Company Act of 1940, which regulates companies that hold most of their assets in the form of securities instead of plants and equipment. "Emerging biotechnology companies need to raise substantial amounts of equity capital to fund research and development", says Carl Feldman, president of the Industrial Biotechnology Association, which petitioned SEC for the exemption. "The fact that R&D-intensive companies temporarily invest these funds ... should not convert them into regulated investment companies." Biotech firms that had been investing solely in US Treasury securities can now purchase higher yielding private bonds. (Source, *C&EN*, 22 March 1993)

FDA rules for somatic-cell and gene therapy

The Food and Drug Administration (FDA, Bethesda, MD) recently issued a statement clarifying how it plans to regulate somatic-cell-therapy and gene-therapy products. The FDA also convened an advisory committee to consider safety-testing procedures at gene-therapy production facilities because of concerns over the use of certain viral-based vectors in gene-therapy clinical protocols.

The FDA policy statement will help to demarcate areas of agency oversight. Particularly for somatic-cell clinical procedures, it has not been entirely clear what falls within the FDA's jurisdiction.

Some of the regulatory "logistics" for somatic-cell therapy procedures are expected to differ from those involving gene therapy.

The FDA policy statement does not propose departing from current practices when it comes to assigning primary review responsibilities for gene-therapy and somatic-cell-therapy products within the agency.

For its part, the FDA advisory committee focused on the risks involved when virus-based vectors are used to shuttle genes in gene-therapy protocols. Specifically, the committee members reviewed information describing two recent instances when "replication-competent" viruses were detected among vector production lines of viruses that had been engineered not to replicate.

Both cases were detected before causing harm, but the appearance of these replication-competent viruses, which arose presumably through multiple recombinant events, remains surprising. To help assure that such viruses are detected as early as possible, FDA officials now are recommending that manufacturers check both supernatant fluids and pellets of their vector preparations at every step. Meanwhile, FDA scientists say they are contemplating a new round of risk-assessment studies to see whether spontaneously derived replication-competent viruses cause diseases in animals. Agency researchers are still deliberating over what kinds of animals - whether primates or rodents or both - are appropriate for such tests. (Extracted from *BioTechnology*, December 1993)

C. RESEARCH

Research on human genes

DNA fingerprinting

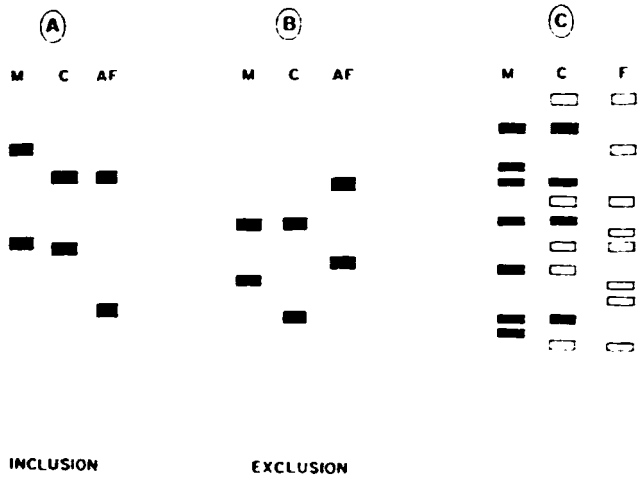
The advent of DNA fingerprinting has opened new vistas in molecular biology. The term "DNA fingerprint" was coined by Prof. Alice J. Jeffreys of the United Kingdom in 1985. Like the conventional fingerprints, this "genetic fingerprint" is also unique to an individual. This uses recombinant DNA technology and has the ability to detect differences between individuals at the level of their DNA.

The genomes of higher eukaryotes, such as humans, consist of certain DNA sequences for which no clear function has been established. Such non-coding "DNA" exists in multiple copies with varying number. These are called the variable number tandem repeats (VNTR). These VNTR regions account for as much as 30 per cent of the human genome. Jeffreys and colleagues isolated a 33bp minisatellite core sequence from the intron of human myoglobin gene and using that, they constructed multilocus probes called 33.6 and 33.15 which detect polymorphism in most of the living organisms (human, animals, plants and prokaryotes). These multilocus probes (MLPs) have homology throughout the genome and show variations between individuals on hybridization to human DNA digested with suitable restriction endonuclease. This results in a business bar code-like band pattern unique to an individual except the monozygotic (identical) twin. The uniqueness of this genetic fingerprint is such that even if the present world population is multiplied five times, there is yet no possibility of finding the same "DNA fingerprint" in any two individuals. Demonstration of Mendelian inheritance, somatic stability and very low mutation rate has enabled this technique to be used in identity testing.

Apart from multilocus probes, single locus probes (SLPs) also play a major role in human genetic analysis by detecting the variations that occur at the single locus or gene level. In the past 10 years an enormous number of single locus probes have been identified. Among the SLPs, VNTR sequences are the most polymorphic ones. Of late, synthetic oligonucleotide probes have also been put to use. The basic unit is a small number of bases (e.g. GTG, GACA, GATA) which are in repeats connected in a head to tail fashion. There are only marginal differences in the DNA typing protocols using cloned probes (SLPs and MLPs) and oligo DNA probes. The protocol essentially comprises: (i) isolation of high molecular weight DNA from the specimen; (ii) restriction of isolated DNA with suitable enzymes; (iii) fractionation of DNA fragments by agarose gel electrophoresis; (iv) fractionation of DNA to nylon/ nitrocellulose membrane (by capillary/vacuum and electroblotting); (v) labelling of probe DNA; (vi) hybridization with the labelled probe; and (vii) interpretation of results through various means.

DNA fingerprinting has a very wide application in the field of medicine, forensic science, demography, animal and plant breeding and biotechnology. In forensic sciences, DNA fingerprinting is used in human identification, rape cases and paternity testing. Paternity testing usually involves the typing of DNA from blood samples of the mother (M), the child (C) and the alleged father (AF). Figures depict three examples (A, B with SLP and C with multilocus probe). Because the offspring receives half of the DNA from the father and half from the mother, each child will receive one allele from each parent. In the case of A, the alleged father (AF) is included as the biological father of the child and in the case of B, he is excluded from being the father of that child (using a single locus probe). The figure C represents the band patterns obtained for a family trio of father (F), child (C) and mother (M) using a multilocus probe.

In medicine, the monitoring of bone marrow transplantation and prenatal diagnosis of certain inherited diseases, such as neurofibronatosis and hereditary persistence of foetal haemoglobingathies are possible due to DNA fingerprinting. Cell line identity and tumour analysis can also be done with this technique. In agriculture, hard and soft wood saplings can be distinguished before implanting. Other areas of application include breeding certain endangered species through proper identification and animal husbandry in veterinary medicine.



FIGURE

Paternity testing by DNA fingerprinting

A & B: Inclusion and exclusion of alleged father (AF). Schematic autoradiograms of the DNA gels are shown respectively with single locus probe.

C: DNA fingerprinting of a family comprising father (F), child (C) and mother (M) using multilocus probe.

AF: Alleged father, C: child; M: mother; F: father

Seminars/Workshops			
15 June - 31 July 1993	Hyderabad	24th Laboratory Animal Technical Training Course 93	S. Haribaran, Assist. Director, Lab. Animals Inf. Service Centre, Nat. Inst. Nutrition, Hyderabad
19-31 July 1993	Bombay	Advanced Training Course in Human Sperm Biology	Dr. K. Gopalakrishnan, Inst. for Research in Reproduction, Bombay
27-29 Sept. 1993	Allahabad	National Symposium on Cell Biology of Parasitic Protozoa	Dr. Dwijendra Gupta, Biochemistry Dept., Allahabad University, Allahabad
11-14 Oct. 1993	Pantnagar	Third Indian Fisheries Forum	Dr. C. S. Singh, Convenor, G. B. Pant, University of Agriculture & Technology, Pantnagar

(Submitted by S. Karuthapandian, Senior Research Fellow, Research & Development Division, Forensic Sciences Department, Madras 600 004 to *Bit.bytes*, Vol. 2, No. 1, 1993)

Colon cancer: gene marker found

An international team of researchers has announced the location of a marker for a gene which predisposes one in every 200 people in the Western world to the disease.

The American and Finnish researchers say the gene makes people susceptible to a form of colorectal cancer that runs in families - up to 15 per cent of all cases of this cancer - and some cases of uterine and other cancers. That would make cancers caused by the gene probably the most common inherited disease yet identified.

The team has yet to pinpoint the gene and sequence it, but by studying families at risk from the disease, they identified a DNA sequence, or marker, on chromosome 2 which is inherited with the gene and so must be close to it.

The researchers suspect the colon cancer gene operates by disrupting DNA replication and causing thousands of other DNA sequences throughout the genome to mutate. The researchers estimate that people who inherit the gene have a more than 90 per cent chance of developing cancer. If caught early, colon cancer is curable in about 90 per cent of cases.

By tracking which family members carry the genetic marker and which develop colon cancer, the researchers can determine who is likely to suffer in future. Those without the marker could be spared what the researchers admit are "costly and difficult" colonoscopies to search for early signs of cancer. (Source: *New Scientist*, 15 May 1993)

Research centre develops new transgenic method to create proteins using fewer genes

A research group under Masashi Kato, Chief Researcher at the Sagami Chemical Research Centre, has developed a new efficient method for recombinant insertion of human genes in animal cells. In the method, a special virus is added to genes replicated in coliform bacteria and implanted with the genes. The virus is then used as a carrier (vector) during recombination in animal cells. The vector is easily extracted from the culture solution, eliminating special refining and concentrating procedures. Proteins can be produced by recombination in animal cells of substantially less than 1/10,000 the present amount of genes used. Experimental periods can be reduced by roughly one week. The method appears promising for understanding gene functions and for research and development of biopharmaceuticals.

The new technology is the product of research by the Kanagawa Academy of Science and Technology (KAST) in the "Human Protein" project. Enzymes were produced by recombining the human gene for production of the enzyme urokinase in coliform bacteria, culturing the bacteria, and introducing the gene into kidney cells from monkeys.

The mixture of a virus known as a helper phage into the culture solution of coliform bacteria during reproduction of the gene played a key role. Once double helical (double chain) deoxyribonucleic acid (DNA) became single stranded, it was taken up by the phage, and when the phage was introduced to animal cells, urokinase was synthesized.

The fragility of single-stranded DNA has made insertion difficult up to the present, but the single-stranded protein of the phage itself is thought to preserve the DNA, allowing insertion without breakage.

The phage is easily extracted, eliminating a special refining process, and large quantities of protein can reportedly be synthesized with a nanogram order (nano = 1 billionth) sample of DNA.

The conventional procedure of extraction and insertion of DNA in double-stranded form results in low rates of expression in animal cells and the need for heightened purity of samples used in protein synthesis. Accordingly, large quantities of DNA must be prepared, and special extraction and refining and concentrating operations have been time-consuming.

Due to their single-stranded DNA structure, helper phages have been used in experiments to determine gene base sequences and the like, but their use as vectors is novel. Why single-stranded DNA is expressed well in animal cells is unknown, but it appears to provide an effective means of gene insertion.

Only a small percentage of all genes whose function provides the basis for protein production are known. Use of the new technology to produce actual proteins from various genes is likely to speed research verifying their functions. (Source: *Nikkei Sangyo Shimbun*, 21 April 1993)

New enzyme for accurate DNA cleavage

A group under Professor Makoto Komiyama of the Tokyo University Faculty of Engineering has developed a method for precise cleavage of DNA at target locations using a synthetic enzyme containing the metal cerium. The development should prove a powerful tool in genetic engineering.

Technology for cutting and rejoining chemically stable DNA is essential in genetic engineering. However, natural enzymes (restriction enzymes) typically employed as "scissors" can only be used for cleavage at limited locations. Meanwhile, precise cleavage has not been obtained with conventional synthetic enzymes employing metallic ions such as iron.

Using an acetic acid compound, Professor Komiyama's group constructed the synthetic enzyme by attaching a cerium ion to the tip of a DNA fragment synthesized to perfectly match the DNA the group sought to cleave.

The DNA fragment binds by recognizing the base sequence of its target DNA. It works by hydrolyzing and cleaving the target DNA with the cerium ion as a catalyst. Since binding can be effected at any location by changing the base sequence of the DNA fragment, sections restriction enzymes cannot cut can also be cleaved.

At the current stage, cleavage on either side of target sites still results due to minute vibrations of the acetic acid compound component, which corresponds to a hand holding the scissors. The research group is thus moving forward with experiments in more accurate cutting by attaching the cerium ion to the centre of the DNA fragment.

According to Professor Komiyama, "past synthetic enzymes carried out cleavage by destroying DNA like a bomb, but the new enzyme is sharp, like a knife, and makes a clean cut. Besides its usefulness to the human genome project under way world-wide, we can expect applications in areas such as treatment of cancer or viral illnesses, where genes are intimately related".

Broad practical application

According to Assistant Professor Hiroshi Sugiyama of the Kyoto University Faculty of Engineering (Synthetic Biochemistry), conventional synthetic enzymes often employ oxidation reactions for cleavage, and the new enzyme is unique for its hydrolysis, identical to that of natural restriction enzymes. If practicalization is achieved, its application will be broad. The need now is a means to achieve reliable cleavage at target sites alone. (Source: *Nihon Keizai Shimbun*, 6 June 1993)

Genetic link to osteoporosis

Drugs to prevent or treat osteoporosis, a degenerative bone condition that affects nearly 20 million Americans - mostly women - could be a step closer to the marketplace, thanks to studies being conducted at the Southwest Foundation for Biomedical Research in San Antonio. Scientists there are using baboon models - whose physiology and genetics are very similar to humans - to pinpoint the genetic causes of the crippling disease associated with ageing. By identifying the genes that control bone mass, scientists hope to develop new therapeutic drugs. Southwest Foundation, home to the world's largest colony of research baboons, is an established leader in using non-human primates to study complex human diseases. (Source: *BioBytes*, November 1993)

Gene therapy trial

Targeted Genetics Corp. has started a gene therapy trial using genetically modified cells that specifically recognize and destroy HIV-infected cells. The trial is a potential treatment for HIV and will evaluate the safety and antiviral effects of HIV-specific cytotoxic "killer" T-cells in HIV positive participants. The modified T-cells also contain a so-called suicide gene which acts as a tracking device and safety valve. This helps direct the killer cells

to their target and provides a means of eliminating the modified cells if toxic side-effects occur. (Source: *European Chemical News*, 8 November 1993)

Australians claim HIV advance

Australian scientists have revealed that they have successfully cloned and mapped the gene for a molecule recently identified by scientists at the Pasteur Institute as being a key that lets HIV enter human cells.

Geoff McCaughan, associate professor at Sydney's Royal Prince Alfred Hospital, believes that the map of the gene for the co-receptor molecule dubbed CD-26 will help identify variations which could help explain why people develop the disease at different rates. Moreover, if CD-26 is the key, then the map may allow scientists to block key parts needed for expression and reduce the risk of HIV infection.

Meanwhile, a team of scientists at France's Laboratoire de Pharmacochimie Moléculaire et Structurale have announced that they have established the three-dimensional structure of an internal protein of the AIDS virus HIV-1. The nucleoprotein 7 plays a crucial role in the reproduction of the retrovirus.

In cooperation with the Lyon-Inserm Retrovirology Laboratory and Rhône-Poulenc Rorer, they are now trying to find the antagonistic molecules which will block the functions of the protein and therefore replication of the virus. (Source: *European Chemical News*, 8 November 1993)

Catalytic antibodies

Catalytic antibodies that promote the breakdown of cocaine before it reaches the brain have been developed by researchers at Columbia University. Although further work is needed, the artificial enzymes represent a new concept in anti-addictive medicine, and the first potential medical application of catalytic antibodies.

The anticocaine antibodies were developed by Donald W. Landry and co-workers in the department of medicine at Columbia University's College of Physicians and Surgeons.

In the study, Landry and colleagues immunized mice with a phosphonate ester whose structure is analogous to that of the putative transition state in the benzoyl ester hydrolysis of cocaine. Immunizing mice with an immunogenic version of this transition-state analog causes the animals to develop antibodies that bind and stabilize the transition state, thus catalyzing the hydrolysis reaction.

The researchers found that two monoclonal antibodies derived from the antibodies produced by the immunized animals can speed up cocaine hydrolysis by a factor of 100 to 1,000. The hydrolysis yields two products - ecgonine methyl ester and benzoic acid, which lack cocaine's stimulant activity.

Currently, there are no direct antagonists of cocaine (drugs that block uptake of cocaine at brain receptors). Hence, drug treatment of cocaine addiction has focused up to now on antidepressants.

To improve the enzymes, Landry, in collaboration with Columbia biochemistry and molecular biophysics professor Wayne A. Hendrickson, is currently trying to obtain their *X*-ray crystal structures, which will help provide a basis for site-directed mutagenesis to enhance the enzymes' activity. (Abstracted with permission from *Chemical & Engineering News*, 29 March 1993, p. 4. Copyright (1993) American Chemical Society)

Poisonous peanuts linked to liver cancer

A link between contaminated peanuts and liver cancer has long been suspected but the reason has remained elusive. Now a group of Swiss scientists has found the connection. Their discovery could be a step on the road to preventing the cancer.

A fungus, which grows on peanuts and produces a toxin called aflatoxin B1 (AFB1) is common in parts of Africa and Asia. Epidemiological studies have suggested the toxin plays a role in liver cancer.

Two years ago, researchers in the United States found that half the liver cancers in these areas have a specific mutation in the tumour suppressor gene, *p53*. Now Peter Cerutti and his colleagues at the Swiss Institute for Experimental Cancer Research have provided the explanation. When they added AFB1 to cultures of human liver cells, they found the very same mutation in the *p53* gene.

A change in codon 249 of the *p53* gene causes a defective protein to be made which may be unable to regulate cell growth and so allows tumours to grow.

Cerutti's *in vitro* work showed that the toxin also produced changes at either side of codon 249 - mutations not seen in human liver cancers *in vivo*. Cerutti says this could mean the tumour selects the genetic mutation which gives it a growth advantage.

Cerutti says the mutation cannot be strictly necessary because it is present only in half the cancers in these parts of the world.

Studies suggest that the effect of the defective gene is amplified if the person also has hepatitis B. People infected with this disease have a four- to fivefold increase in their risk of developing liver cancer. People exposed to AFB1 double their risk. People with both are 50 times more likely to develop liver cancer.

Cerutti's team is examining liver tissue from people in the Far East, to determine whether the mutations are present long before the cancer or whether a mutation leads to tumour growth almost immediately.

Cerutti believes it may one day be possible to screen people for the genetic mutations. Preventative measures could then be taken. (Source: *New Scientist*, 25 September 1993)

Huntington's disease

A consortium of scientists has discovered the HD gene on chromosome four. Identification of the gene is expected to lead to new specific diagnostics enabling members of families at risk for the disease to be informed that they do or do not carry the mutation and will or will not eventually develop the disease. The discovery was made by a large collaborative group from the University of California, Massachusetts General Hospital, the Hereditary Disease Foundation in Santa Monica, University of Michigan, Massachusetts Institute of Technology, University of Wales College of Medicine and the Imperial Cancer Research Fund, London. (Source: *Australasian Biotechnology*, Vol. 3, No. 3, June 1993)

Scientists solve protein structure that could lead to anti-cancer drugs

A team of researchers led by Drs. Steven Reed and John Tainer at the Scripps Research Institute (La Jolla, CA) has solved the atomic structure of a protein that may lead to the design of a new class of anti-cancer drugs. The protein, known as CksHs2, is a cell cycle regulatory protein that controls cell division at two critical points - immediately preceeding DNA synthesis and cell division. Using X-ray crystallography, the scientists were able to determine how the protein's structure allows it to have a central role in regulating the cell cycle which controls cell division.

Because malignant cells are defective in at least some checkpoint controls, the protein may afford a primary target for the design of drugs to exploit this intrinsic vulnerability. The researchers have identified three specific regions predicted to be biologically important interaction sites that are priorities for testing by site-directed mutagenesis and targeted drug design. (Source: *Genetic Engineering News*, 1 November 1993)

New type of gene abnormality plays important role in cancer

For almost 100 years, scientific theory has held that individuals inherit working copies of genes from each parent and that when one of the two copies malfunctions, it can lead to illness. New research at the Howard Hughes Medical Institute at the University of Michigan Medical Center (Ann Arbor), however, shows that some genes defy these laws of inheritance. For these genes, it is normal for only one copy to work, and two active copies may cause disease.

Dr. A. Feinberg and his colleagues have identified genes that "remember" which parent they came from and either function or turn themselves off, depending on their sexual origin. Such imprinting has been identified in other species, but never demonstrated in humans before, said Dr. Feinberg. His team also found one gene that seems to trigger childhood tumours when copies from both parents work. This gene, which makes insulin-like growth factor 2, loses its imprinting in Wilms' tumour patients. The female-derived gene that is supposed to be silent was working in the cancer cells.

The discoveries of imprinting genes in humans and the gene that appears to trigger Wilms' tumour hint that it may some day be possible to treat cancer by switching one gene back to its normal silent state. "Our data suggest that relaxation of imprinting may be a first step in cancer. It causes overexpression of this growth factor so tumours grow", said Dr. Feinberg. "A large number of childhood tumours show increased IGF2 expression, and it is thought to be important in breast, colon and lung cancers in adults. We know that if you block IGF2 expression, some tumours do not grow." (Source: *Genetic Engineering News*, 15 May 1993)

Antibody binding in lupus causes changes in DNA

Anti-DNA antibodies in mice prone to lupus force structural changes in DNA upon binding, a mechanism that may be related to the pathogenesis of the disease. Lupus is an autoimmune disease in which anti-DNA antibodies bind to DNA. The resulting complexes become lodged in the kidneys of humans and mice, where they trigger inflammatory reactions that can lead to kidney failure. Gary D. Glick and Shawn Y. Stevens of the University of Michigan, Ann Arbor, and co-workers have now used electrophoretic assays to monitor the interaction between anti-DNA antibodies and segments of DNA that have a hairpin loop structure, a known recognition site for antibody binding in lupus. The hairpin loop contains double-stranded DNA connected to a segment of single-stranded DNA. Glick and co-workers found that lupus antibodies bind to the single-stranded DNA and then "melt" the adjacent double-stranded segment, producing a larger single-stranded region. In future work, they hope to identify the exact structural features of DNA targeted by lupus antibodies. (Reprinted with permission from *Chemical & Engineering News*, 1 March 1993, p. 24. Copyright (1993) American Chemical Society)

Scientists discover location of gene for glaucoma

Opthalmology researchers at the University of Iowa (Iowa City) College of Medicine have discovered the location of a gene for an inherited form of glaucoma. Edwin Stone and his team used chromosome linkage analysis to map the gene to the long arm of chromosome

one (with an odds ratio of one million to one). Their work was accelerated by information from a man who noted that five generations of his family were affected with severe glaucoma. The researchers, who found that 19 of 37 family members had elevated pressure in the eye and detectable loss of vision, are now searching this portion of chromosome one for the individual gene that causes the disease. (Source: *Genetic Engineering News*, 15 May 1993)

Pasteur Institute testing disease-fighting cells

A team of researchers at the Pasteur Institute has developed a new gene therapy technique consisting of "pouching" and introducing genetically modified cells directly into the body of a diseased mouse, and not, as is current practice, into the diseased organ.

According to the researchers (Philippe Moullier, Delphine Bohl, Jean-Michel Heard, and Olivier Danos, of the Retrovirus and Gene Transfer Laboratory), "this approach produced a remarkable improvement in the condition of the animals". Philippe Moullier, the inventor of this technique, which oscillates between gene therapy and organ transplants, explains that the cluster of cells introduced into the body of the diseased mouse secretes the missing protein continually, throughout the body, and not just in the diseased organ, where the diffusion is only local.

The animals selected by the Pasteur Institute team were afflicted with a genetic disease that prevents cells from ridding themselves of their waste. This anomaly very rapidly affects all the body's tissues. In the case of the mice used at the Pasteur Institute, the enzyme deficiency involved entails lesions of the various organs (liver, spleen, brain) and bone deformations.

Such diseases, linked to the absence or dysfunction of the enzymes that participate in the cellular "tidying up" process, exist also in human beings. These diseases are known as lysosomal storage diseases - a group that includes Gaucher's, Tay-Sachs, and Hurler's among others. They are relatively rare but can be very serious from very early childhood.

To carry out their experiment, the researchers withdrew skin cells from the diseased animals, and placed these cells in a culture together with viruses that would serve as carriers whose function would be to introduce the missing gene into the mouse's genetic material. The cells were then mixed with collagen and artificial fibres, and the mixture, a sort of pouch filled with cells, was introduced into the abdomen of the mice.

According to the researchers, the results are "striking": the cell mass vascularizes very quickly, the enzyme begins to be produced and to be diffused throughout all the organs. Little by little, liver and spleen lesions clear up "spectacularly". The bone deformations, however, once they have set in, appear to be irreversible. This is why the genetic engineers are now testing their method on newborn mice, in which bone lesions have not yet appeared and in which the enzyme, moreover, appears to have better access to the central nervous system.

"This research", says the Pasteur Institute, "is of great interest to clinicians who have no means of treating these diseases other than by very hazardous bone marrow transplants and, in some cases, extremely costly enzymatic therapies". Use of the technique being developed by this research could moreover not be limited to the treatment of lysosomal storage diseases, but could also be applied to other genetic diseases, particularly those for which medicaments of proteinic origin are used, such as insulin for diabetes, and factor VIII for haemophilia.

Before beginning any testing whatever on humans, the researchers must carry out tests on animals of larger size than mice in order, on the one hand, to verify that this technique can be used on them and, on the other hand, to determine whether the therapeutic effect produced in the mice will be produced in other animals as well. (Source: *AFP Sciences*, 15 April 1993)

Cloning and expression of human CD4 gene in *E. coli*

CD4 molecule is an important differentiation antigen of T lymphocyte and has been identified as the receptor for HIV. Ji Changhua, Su Chengzhi, et al. of the Department of Biochemistry, The Fourth Military Medical University, Xi'an have designed and chemically synthesized two primers and successfully amplified a gene fragment encoding the N-terminal two domains of human CD4 protein. EcoRI and Hind III recognition sites and the initiation and termination codons were incorporated into the 5' and 3' termini of this gene through polymerase chain reaction The CD4 gene fragment was digested with EcoRI and Hind III and inserted into pUC19 plasmid. The recombinant clones were confirmed by polymerase chain reaction and restriction endonuclease cleavages and one of the correct clone pT403 was sequenced by dideoxynucleotide termination method and it revealed that the sequence of the cloned gene fragment was identical to that of the published CD4 cDNA. The CD4 gene was then inserted into the prokaryotic expression plasmid pSM43 and transformed TAP106 bacteria, through induction at 42° C, a new protein band appeared in the recombinant bacteria as seen on SDS-PAGE gels. The expression reached its highest level at five hours' induction when the CD4 protein accounted for 24.8 per cent of the total bacterial proteins. By a few steps of purification, the CD4 protein was shown as a single band on SDS-PAGE. (Source: *Chinese Journal of Microbiology and Immunology*, Vol. 13, No. 7, October 1993)

RNA ATP-binding motif found by *in vitro* selection

A small RNA sequence has been found that binds ATP, a frequent participant in biological reactions and the universal medium of biological energy storage and exchange. The work was done by Mandana Sassanfar and

Jack W. Szostak of the Department of Genetics at Harvard Medical School. They used *in vitro* selection - a technique involving construction of a large pool of random polynucleotide sequences, followed by repeated cycles of enrichment for species with a desired property and amplification of the enriched species. The RNAs selected for ATP binding contain an 11-base consensus sequence and adopt a common secondary structure. "The ATP-binding motif and other RNA structures capable of binding small molecules in solution could serve as building blocks for the construction of a new set of ribozymes with a wide range of catalytic activities", the researchers say. (Reprinted with permission from *Chemical & Engineering News*, 9 August 1993, p. 15. Copyright (1993) American Chemical Society)

Custom-built traps catch DNA bases

Molecular receptors that distinguish between the four DNA bases - adenine, cytosine, guanine and thymine - have been designed by chemists in Canada. The receptors are unique because they exploit the three kinds of molecular interaction these bases respond to. The chemists' work has implications for molecular biology and genetics, and especially for understanding the working of anti-cancer drugs such as the platinum-containing cisplatin, which seeks out particular combinations of bases along strands of DNA.

Stephen Loeb, James Kickham and Shannon Murphy of the University of Windsor, Ontario, prepared several receptor molecules. One is a ring of atoms incorporating two sulphur atoms, four oxygen atoms and three benzene rings. Sulphur atoms can bind to metal atoms and oxygen atoms can form hydrogen bonds. The chemists also made other receptors without the benzene rings, or without the oxygen atoms.

The receptors were completed by inserting a palladium atom into them. This involved dissolving the molecule in a solution of a palladium salt and the solvent acetonitrile. The metal bound itself to the sulphur atoms of the receptor molecule. Although the metal had a molecule of solvent clinging to it, this was easily displaced by a base.

The next step was to see which of the bases would fit the receptor. Loeb's team tested these by simply adding the base, in solid form, to a solution of the receptor and vibrating this with ultrasound for 15 minutes. They then filtered the mixture. Nuclear magnetic resonance spectroscopy revealed whether or not the base had been trapped by the receptor.

The Canadians tested how well their receptors could distinguish between the four bases. Some were particularly good at targeting one or other of them. Adenine and guanine were attracted most strongly to the rings which had both oxygen atoms and benzene rings. Cytosine preferred a ring with only oxygen atoms. Thymine, on the other hand, could not be enticed into any of the rings. The chemists expected this because thymine lacks the nitrogen atoms used by the other bases to bind to palladium.

The chemists grew crystals of the guanine-receptor combination. X-ray analysis revealed how the two are held together by the three types of bonding. First, the guanine bonds strongly to the palladium through one of its nitrogen atoms. Secondly, it is attracted to two of the oxygen atoms of the ring by hydrogen bonding of its amino (NH_2) group at the other end of the molecule. Thirdly, it is held in place by what is called π-π stacking. This attraction occurs between the electron cloud above and below the guanine molecule and similar electron clouds of the benzene rings.

Until now, the incorporation of bases into molecules has always been based on their strong attraction to metal atoms. Loeb and his colleagues have now shown that weaker, more subtle, interactions can also be used to trap these bases and, more importantly, to distinguish between them. Loeb's group is now studying the application of their receptor molecules to biological systems. (Source: *New Scientist*, 4 September 1993)

Protein may boost sensitivity to cisplatin

A new study has disclosed a potential route to extending the applicability of the anti-cancer drug cisplatin to different types of tumours. Cisplatin is effective in chemotherapy of testicular and ovarian cancer, but targets other cancers less specifically. The drug works by binding to DNA and generating intrastrand cross-links, which block replication and cause cell death. Earlier, chemistry professors Stephen J. Lippard and John M. Essigmann of Massachusetts Institute of Technology and co-workers identified endogenous proteins in human cells - called structure-specific recognition proteins, or SSRPs - that bind to DNA containing cisplatin cross-links. The function of SSRPs is not yet clear, but one theory is that they shield intrastrand cross-links from DNA repair enzymes and therefore tend to enhance cisplatin's cytotoxic effect. Lippard and post-doctoral associates Steven J. Brown and Patti J. Kellet have obtained results that tend to confirm that theory (although the results do not rule out other possible mechanisms). The researchers find that an SSRP-deficient yeast strain shows only a third as much cisplatin-induced cross-linking and is only half as likely to be killed by cisplatin as a normal strain. The results suggest, they say, that tumour cells might be sensitized to cisplatin if levels of SSRPs in the cells could somehow be increased. (Reprinted with permission from *Chemical & Engineering News*, 9 August 1993, p. 18. Copyright (1993) American Chemical Society)

Structure of muscle protein myosin determined

The 3-D structure of the portion of myosin that generates motion in muscles has been determined by researchers at the University of Wisconsin, Madison, and the Robert Wood Johnson Medical School, Piscataway, NJ. The team was headed by Wisconsin crystallographer and

molecular biologist Ivan Rayment, who has been working on the project for 10 years. "It took almost six years to work out a way to get good crystals", he notes. "It took another three years to solve the crystal structure." The Wisconsin group also teamed with researchers from Scripps Research Institute, La Jolla, CA, and Max Planck Institute for Medical Research, Heidelberg, Germany, to propose a new model of how the proteins myosin and actin (whose structure is known) produce the sliding motion within cells that results in muscle contraction. The work shows that the myosin head is an elongated, pear-shaped molecule that bends in the middle. It had been thought that the head was rigid and pivoted to propel the filaments past one another. It now appears that the wide part of the myosin head has a jawlike structure that closes, binding the myosin to the actin. The myosin head then flexes in the middle, pushing the myosin filament in one direction relative to the actin filament. The cycle then repeats. (Reprinted with permission from *Chemical & Engineering News*, 5 July 1993, p. 19. Copyright (1993) American Chemical Society)

Technique permits DNA sequencing by hybridization

A key milestone has been passed for a promising DNA sequencing technique that could potentially be used to speed mapping and sequencing of the human genome. The technique - sequencing by hybridization (SBH) - uses an array of all possible DNA oligomers containing a set number of nucleotides to identify same-length sequences present in an unknown DNA. Identification is based on hybridization of labelled oligomers with support-bound DNA, or hybridization of labelled DNA with support-bound oligomers. Three DNA fragments with a combined length of 343 base pairs were sequenced accurately, using an array of eight-nucleotide DNA oligomers. SBH has been under development for six years, but this is the first time it has been proved capable of error-free sequencing in a double-blind test. The work was done by biologists Radomir Crkvenjakov, Radoje Drmanac and colleagues at Argonne National Laboratory, and Leroy Hood (now at the University of Washington) at the California Institute of Technology. SBH, which can be automated and multiplexed, could eventually prove to be faster at genomic sequencing than conventional gel sequencing techniques. Other likely applications include putting overlapping DNA clones in the correct order (physical mapping), error-checking DNA sequences generated by other techniques, comparing DNA fingerprints of normal and disease-causing genes, and identifying DNA fragments with specific sequences from large libraries. (Reprinted with permission from *Chemical & Engineering News*, 14 June 1993, Copyright (1993) American Chemical Society)

Modified RNA precisely cleaves target DNA

RNAs containing modified uridine triphosphate (UTP) nucleotides linked to the chemical nuclease, 1,10-phenanthroline-copper(I) efficiently cleave single- and double-stranded DNA targets sequence specifically. David S. Sigman, a biological chemistry professor at the University of California School of Medicine in Los Angeles, and colleagues Chi-hong B. Chen and Michael B. Gorin enzymatically produce RNA complementary to a target DNA sequence using 5-(3-aminoallyl)-UTP as the sole source of UTP. They use a cross-linking reagent to attach 1,10-phenanthroline to the aminoallyl-UTPs in the RNA. In the presence of copper, which coordinates to the phenanthroline group, this reagent efficiently cleaves single-stranded DNA sequence specifically. Three-stranded structures known as "R loops" provide the method of sequence recognition of double-stranded DNA. These structures form when RNA displaces the segment of DNA of identical sequence in the presence of high concentrations of formamide to form an RNA-DNA duplex. The strategy developed by the UCLA chemists allows cleavage of DNA at any chosen sequence, Sigman points out, and is likely to facilitate the analysis of chromosome and gene structure in a variety of ways. (Reprinted with permission from *Chemical & Engineering News*, 3 May 1993, p. 42. Copyright (1993) American Chemical Society)

Research on animal genes

Heat-shock protein exhibits protection against tumours

Immune-response therapies to treat cancers, or even to protect people from developing cancer, might stem from findings at the National Institute for Medical Research in London, an arm of the United Kingdom's Medical Research Council. That is the conjecture of M. Joseph Colston, head of the Laboratory for Leprosy and Mycobacterial Research, and co-workers, arising from their studies with heat-shock proteins (hsp). On transferring the gene encoding the highly immunogenic mycobacterial hsp65 into a murine tumour cell line, the researchers find the resulting hsp65-expressing cells are incapable of inducing tumour production in mice specially bred for the susceptibility to cancer. Moreover, similar mice immunized with the impotent cells do not develop tumours when challenged with the parent cancer cells. "Results demonstrate a highly significant level of protection", Colston and his colleagues note. One group of immunized mice were healthy six months after being challenged with cancer cells, and 80 per cent of them survived more than a year, they add. (Reprinted with permission from *Chemical & Engineering News*, 28 June 1993, p. 24. Copyright (1993) American Chemical Society.)

New transgenic mouse hybrid

Genpharm International and Stratagene have developed a transgenic mouse for mutagenesis and tumourgenesis research. A cross between GenPharm's *TSG-p53* transgenic mouse and Stratagene's *Big Blue* transgenic mouse, the new mouse will enable scientists to conduct - for the first time - tissue-specific *in vivo* testing for mutations and tumours in the same animal.

The *TSG-p53 Big Blue* transgenic mouse will be marketed with Stratagene's mutagenesis assay system.

It has been estimated the GenPharm's *TSG-p53* model will have a turnover of $5 million/year. Stratagene's *Big Blue* could see global sales of some $80 million. Sales of the hybrid are anticipated at $10 million. (Source: *European Chemical News*, 14 June 1993)

Combination of mouse interleukin receptor antibody with human antibody

Chugai Pharmaceutical Co., Ltd. (President: Osamu Nagayama) and Professor Chuzo Kishimoto (Osaka University, School of Medicine) have successfully combined a mouse interleukin receptor antibody with a human antibody gene. Based upon the results of their study using an animal model, they have confirmed that the resultant product has an anti-cancer effect. Interleukin-6 (IL-6) is among those factors that cause the proliferation of multiple myeloma. IL-6 binds to the receptor of a cell, thus leading to cell proliferation. These researchers have produced an antibody against the receptor for IL-6 in mice, and the company plans to begin clinical trials.

Multiple myeloma, a cancer in bone marrow, is known to occur simultaneously in many different regions of the body. Chugai Pharmaceutical Co., Ltd. has devoted its efforts toward producing an antibody against the cell receptor site of IL-6. As the antibody binds to the receptor, it follows that IL-6 is unable to bind to the cell, thereby suppressing the proliferation of multiple myeloma. Because it was not possible to produce the antibody from a human system, it was produced instead in mice.

Although mouse antibodies PM1 and AUK12-20 bind to human IL-6, the human system produces an antibody against the mouse antibody. To prevent such occurrence, modification of the mouse antibody suited for humans is essential.

Chugai has managed to combine a human antibody gene with a mouse antibody gene that recognizes antigen. Technical assistance in conjunction with this research effort was rendered by experts from the Medical Research Centre in the United Kingdom, who engaged in a study involving the crystal structural analyses of antibodies of humans and mice.

The combination of mouse antibody with a human antibody gene was shown to have the same effects as the mouse antibody alone in terms of binding strength and ability to suppress the proliferation of multiple myeloma with antigen. Nude mice were used in assessing the ability of the human-type antibody to cause such suppression. The human-type antibody gene PM1, in dosages of 0.125 mg per mouse, was injected three times; the induced tumours were weighed 56 days thereafter. The results showed that said tumours weighed only 2-3 per cent of those without injection. According to the company, this result confirmed the anti-cancer activity.

In the treatment of multiple myeloma, two different types of therapy might be applied: one that uses the antibody against IL-6 and another that uses the antibody against the IL-6 receptor site. This company and Professor Kishimoto began their cooperative research venture on receptor genes; ultimately, they have succeeded in producing a combination of mouse antibody with a human antibody gene.

It is fully anticipated that this newly-discovered antibody will be used as a therapeutic agent in treating myeloma that involves IL-6. Chugai Pharmaceutical Co., Ltd. hopes to engage in clinical tests this year following the safety tests of this antibody. (Source: *Nikkan Kogyo Shimbun*, 3 May 1993)

Baleen whale's genes capture echoes of past

The classification of whales and dolphins (order *Cetacea*) should be completely revised, say researchers from the United States and Belgium who have analysed the DNA of these animals. According to the new analysis, the sperm whale and the pygmy sperm whale, both of which have teeth, are more closely related to baleen whales than other toothed whales.

Ideally, the classification of a group of animals should reflect how the animals evolved. Fossils can give an indication of this but usually the classification is based on the identification of common characteristics. Whales and dolphins were divided into two suborders. The toothed whales (*Odontoceti*) have teeth and use echolocation, whereas baleen whales (*Mysticeti*) have baleens - plates in the mouth - which filter food from the water. Sperm whales were grouped with the *Odontoceti* because they have teeth in their bottom jaw.

Now Michel Milinkovitch of the Free University of Brussels and his colleagues at the State University of New York have found that this classification is not compatible with their DNA analysis of several whales and dolphins.

The researchers studied 16 cetacean species. They determined the DNA sequences of the genes that coded for the mitochondrial 12S and 16S ribosomal RNA, and compared them with the aid of a computer. They found that the DNA sequence of the sperm whale and pygmy sperm whale were more closely related to the baleen whales than to other toothed whales and dolphins. The study also showed that the beaked whale (which has only a few teeth) is only distantly related to any other whale or dolphin.

To check their results, the researchers obtained from a database the DNA sequences of the myoglobin of 10 whales and dolphins. Myoglobin is the protein that helps muscles to take up and store oxygen. Again, the researchers found that the two sperm whales were more closely related to the baleen whales, and that the beaked whales were distantly related.

The findings have important implications for the evolution of echolocation. According to traditional classification, the ancestor of the toothed whale developed echolocation, while the baleen whales never had the ability. But the revised classification implies that the ancestor of all whales used echolocation, and that the baleen whales subsequently lost the ability. The alternative explanation, that both the sperm whales and other toothed whales developed echolocation independently, is unlikely. (Source: *New Scientist*, 20 February 1993)

Insecticide resistance

Researchers at the University of Wisconsin have decoded the gene that is responsible for resistance to a major class of insecticides.

The team has been trying to find out how insects become immune to the cyclodiene family of pesticides, which act upon the receptors for the neurotransmitter γ-aminobutyric acid (GABA), interfering with the insects' central nervous system. Working with a resistant strain of the fruit fly *Drosophila*, the team has managed to clone the mutated GABA receptor and locate the area of the receptor responsible for the resistance.

The team sequenced DNA from resistant and non-resistant flies, and tracked the mutation, which is a single switch in the amino acids sequence. "It just happened to be on the GABA receptor", says team leader Richard ffrench-Constant. Cyclodiene pesticides work by binding strongly to the receptor and blocking the GABA molecule, he explains. In resistant flies, the pesticide-receptor interaction is 100 times weaker, so the pesticide is easily displaced, rendering it useless.

Cyclodiene resistance is extremely common, according to ffrench-Constant - in fact, it may account for almost two thirds of all resistance cases. The team has found an identical mutation in resistant yellow fever mosquitoes, and is checking the genomes of other insects.

This discovery could have important commercial implications, says ffrench-Constant. The team has developed a technique which allows it to "screen" the insect population in a particular region to determine the extent of resistance. It can then advise the region's farmers as to which pesticides are most likely to be effective. The team has applied for a patent for this technique, he says, and hopes to be able to sell it to pesticide manufacturers. Its next target is the gene conferring resistance to pyrethroid insecticides. Eventually, the team hopes that its discoveries might put a stop to what ffrench-Constant describes as "spray and pray" testing. (Source: *Chemistry & Industry*, 21 June 1993)

DNX biotherapeutics claims pig organ transplant breakthrough

Heralding a "breakthrough" in the genetic engineering of pigs to provide organs for transplantation to human patients, researchers from DNX Biotherapeutics said they had bred animals with human genes that would prevent their organs being rejected by the patient's immune system after transplant. Dr. John Logan, head of research, said that kidneys and hearts were likely to be used first, and that human clinical testing could begin within 3-5 years. (Source: *Biotechnology Bulletin*, October 1993)

Research on plant genes

Crab apples give clues to insect resistance

Cornell University (Ithaca, NY) scientists report finding a natural source of insect resistance in crab apples. The researchers say the work could eventually allow them to incorporate genes responsible for the insecticidal activity into other apples through conventional breeding or recombinant DNA techniques. The apples resist apple maggots, a common pest, by inhibiting the enzyme cholinesterase in the insects. While the scientists have not yet isolated the specific insecticidal compound, they say its mechanism of action is similar to organophosphate and carbamate insecticides. (Source: *Chemical Week*, 18 August 1993)

Fungus pulls antibiotics from rice

Research by a team at the National Institute of Agro-Environmental Science (NIAES), a research arm of Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF), has shown that an oligosaccharide derived from a fungal polysaccharide induces rice plants to produce the antibiotic phytoalexin.

In experiments using cultured rice cells, the team found that extremely low concentrations of the compound induce production of the antibiotic. Moreover, the oligosaccharide does not appear to have any effect on other plants. The team plans to genetically engineer rice plants to contain the gene for production of the compound to develop a new disease-resistant strain. (Source: *McGraw Hill's Biotechnology Newswatch*, 5 July 1993)

All in the best taste?

Filter-tipped cigarettes reduce the amount of nicotine that is inhaled, but some smokers dislike them because they also trap other ingredients of the smoke, spoiling the flavour. Far better, suggest Thomas Davis of California and Holly Marcun of Maryland, to produce tobacco plants genetically altered to produce less nicotine.

The tobacco plant synthesizes nicotine by a complex series of reactions which start with ornithine and nicotinic acid. Davis and Marcun's technique, patented by Technology Management Services of Switzerland in WO 93/5646, disrupts the biosynthesis by blocking the action of the enzymes that induce the reactions.

The technique introduces modified segments of nucleic acid from the gene that produces the nicotine

demethylase enzyme. This is done either by direct injection with micropipettes or by infecting the plant with a bacterium such as *Agrobacterium tumefaciens* carrying the modified gene. Once the plant has been successfully modified, it can be bred or cross-bred naturally.

The inventors claim that leaves from the modified plants retain all the ingredients that give tobacco its taste and smell, but contain less nicotine. (Source: *New Scientist*, 28 August 1993)

Blight-fighting genes enlisted in battle to save chestnut and elm trees

A team at the State University of New York's forestry college in Syracuse is trying to create an artificial gene that will give the once-treasured chestnut tree a defence against blight.

And another team - at the University of Toronto - is working on a "vaccine" for Dutch elm disease, using genetic engineering to create a harmless fungus that will induce an immune reaction before the tree is invaded by a wild pathogen.

Charles Maynard and colleague William Power of SUNY have found peptides - some from the snail *Helix pomatia* and others from the African clawed frog - that show anti-fungal activity *in vitro*. Their plan is to use the natural anti-fungals as a template and create what Powell calls "an optimal sequence" - an artificial fungus-killing peptide.

In Toronto, Martin Hubbes is attacking Dutch elm disease from a different angle; instead of engineering the tree, he is working on the pest - a fungus called *Ophiostoma ulmi*.

O. ulmi has two major subgroups - an aggressive strain that kills elms very quickly and a less virulent strain, which destroys the trees more slowly. Elms have defences against the fungus, but they seem to work well only against the less virulent strains.

One reason may be that the aggressive strains produce high levels of a toxin called cerato-ulmin, which Hubbes and his colleague, Paul Horgen, believe is a key to their virulence.

By working backward from the structure of the toxin, they found and blocked the gene responsible, creating a strain of fungus that cannot express the toxin. Now, they hope to use this de-fanged fungus to produce an "immune reaction" against the wild type. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 20 December 1993)

Research on yeast and fungus genes

Total base sequences determined for yeast's chromosome 6

The Tsukuba Life Science Centre of the Institute of Physical and Chemical Research (RIKEN) has determined jointly with the Fujiya Bioscience Research Institute about 280,000 total base-sequences of chromosome 6 of yeast as a model for the human genome. The research elucidated that there exist 210 or more genes in the chromosome including about 60 per cent of an unknown one.

Genetic information which maintains the life activities of a living organism and transmits them to its descendants is contained in chromosomes which exist in a nucleus of a cell. Man has 24 kinds of chromosomes, in each of which DNA (deoxyribonucleic acid), the substance of the gene, is finely designed.

In the case of the human genome, genetic information is incorporated into DNA with four kinds of base sequences. This information is contained in the gigantic amount of 3 billion base pairs. Accordingly, genome analysis has been conducted by using model materials such as yeast, which has 16 million base pairs - about 1/200 of man's, and a nematode which has 100 million base pairs - 1/30 of man's.

Among the 16 kinds of yeast chromosomes, the total base sequences of chromosome 3 had been determined by a research group of the European Community. Following this effort, Japanese researchers determined about 280,000 base sequences of chromosome 6 this time.

(For further information, contact the Planning and Research Section, Tsukuba Life Science Centre, RIKEN, Japan; telephone 0298-36-9136.) (Source: *STA Today*, 1 February 1994)

Research on viral genes

Phase 3 testing for anti-CMV human monoclonal antibody

Teijin Ltd. is proceeding with the clinical development of a new drug TI-23 (development number) to treat diseases caused by cytomegalovirus (CMV) infection. The drug will soon by ready for phase III clinical testing. The drug is an anti-CMV human monoclonal antibody developed by Teijin, and currently is in the final stages of phase II testing for prevention and treatment of CMV infection in organ transplant patients. It is expected to move up a step to phase III with the aim of entering the market in a few years. The anti-CMV antibody is under clinical development at Teijin for the

treatment of AIDS patients and organ transplant patients in the United States. The German company Bayer AG is introducing the technology to Europe.

CMV is a DNA virus belonging to the herpes virus family. The virus is transmitted by blood transfusions, sexual contact, and from mothers to their foetuses, but normally the virus goes undetected. However, when people suffer from immune deficiencies such as AIDS, or are taking immunosuppressants after an organ transplant, the capability of the immune system is compromised, and the immunosuppressing action of CMV itself acts synergistically, resulting in a severe infection. CMV is also known as the agent that causes mononucleosis and congenital cytomegalic inclusion disease, and has also been linked to Kaposi's sarcoma, a typical condition of AIDS patients, and prostate cancer.

Teijin has succeeded in developing a neutralizing antibody for the virus that in the past existed only in minute amounts and could not be manufactured. This neutralizing antibody, which suppresses infectivity, is a human monoclonal antibody that reacts with a neutralizing antigen isolated and purified from a glycoprotein (gp55). Teijin is proceeding with development for clinical application. It now appears that the drug, which is currently in the final stages of phase II clinical testing, will move on to phase III. Teijin will concentrate in the future in developing this drug into a commercial product. (Source: *Kagaku Kogyo Nippo*, 3 June 1993)

Establishment and verification of animal model of immunological tolerance against hepatitis B virus

Xiong Sidong, Zhang Wei, et al. at the Laboratory of Molecular Virology, Shanghai Medical University, Shanghai, China, report that, in order to establish an animal model of immunological tolerance against the hepatitis B virus (HBV), one-day old newly hatched ducklings were experimentally infected by injection of the duck hepatitis B virus (DHBV) positive serum simulating perinatal transmission of HBV. Both DHBs/presAg and DHBV DNA remained positive at 22 weeks' follow-up. ELISA detected neither DHBs/pres antibody nor DHBc antibody in sera viral DHBs/presAg, and DH Bc Ag antigen specific lymphocyte proliferation of DPBLs were also not observed. For further verification, purified DHBs/presAg and DHBcAg were used to immunize the persistently infected ducks. Antibodies and lymphoproliferative responses to DHBs/presAg and DHBcAg were not detected. Thus, an animal model of immunological tolerance induced by DHBV infection in one-day-old ducklings was well established and verified. These results may have implications for the study of immunological tolerance against HV B and future therapeutic strategies. (Source: *Acta Academiae Medicinae Shanghai*, Vol. 20, No. 5, September 1993)

Synthesis and application of hepatitis C virus peptides C_1, C_2 and N_1, N_2

Lu Zhimeng, Zhang Yan et al. at Ruijin Hospital, Shanghai Second Medical University, report that three different peptides derived from immunodominant regions of both core and non-structural proteins based on recognized HCV genome sequences were designed, and a sensitive and specific enzyme-linked immunosorbent assay using these peptides (C_1, C_2, N_1, N_2) as the coated antigen was developed. The specificity of antibodies to these peptides was evaluated by inhibition test and the ranges of inhibition rate were 77 per cent to 100 per cent. The researchers detected 44 serum samples and the results obtained were in agreement with the Abbo., 2nd-Generation Kit in 97.7 per cent of the samples. A total of 1,025 samples from various clinical groups were tested for anti-HCV and the antibodies were detected in 43.9 per cent of chronic NANBH patients, 34.4 per cent of blood dialysis patients, 25 per cent of patients with liver cirrhosis, 17.4 per cent of acute NANBH patients, 16.7 per cent of patients with hepatocellular carcinoma and 2.4-5.4 per cent of volunteer blood donors. The results showed that this assay is of fairly good value in clinical practice. (Source: *Chinese Journal of Infectious Diseases*, Vol. II, No. 3, August 1993)

'Flu virus infection mechanism identified

Scientists at Whitehead Institute, Cambridge, MA, have discovered a springlike mechanism for the infective process by which a 'flu virus gains access to a cell. The findings, which may also be valid for other viruses, could aid in the design of new antiviral drugs. 'Flu infection begins when a 'flu virus binds to the surface of a cell. This causes the cell membrane to fold inward, forming a small bubble (endosome) that pinches off inside the cell and has the virus inside it. The virus then fuses with and bursts the endosome membrane, releasing viral DNA into the body of the cell. Although it was known that the viral protein haemagglutinin plays a key role in the fusion of virus with endosome membrane, the detailed fusion mechanism remained a mystery. Now, graduate student Chavela M. Carr and Professor Peter S. Kim of Whitehead Institute's Department of Biology have developed a novel model for the process. The model proposes that a drop in pH causes a conformational change that releases a "clamp" subunit on haemagglutinin, causing a "fusion peptide" on the protein to contact the endosome membrane, resulting in fusion. Based on the model, says Kim, therapeutic agents might be designed that prevent activation of haemagglutinin or cause the conformational change to occur prematurely. (Reprinted with permission from *Chemical & Engineering News*, 31 May 1993, p. 22. Copyright (1993) American Chemical Society)

'Flu virus inhibitors developed using rational drug design

Researchers have used rational drug design to develop compounds that inhibit replication of the influenza virus in cell culture and in animals. Although the compounds' 'flu-fighting ability has not yet been demonstrated in humans, they could potentially lead to new anti-'flu medicines. The work was done by pharmaceutical chemist Mark von Itzstein of Monash University, Victoria, Australia, and co-workers. The 'flu virus has successfully evaded nearly all therapeutic and preventive efforts, largely because of its tendency to mutate rapidly into new, drug-resistant strains. Hence, Itzstein and co-workers focused on a part of the virus that remains invariant among strains, the active site of sialidase, a glycoprotein enzyme on the surface of the virus. Using computer models of the enzyme, the researchers predicted sialic acid derivatives that would bind tightly to the active site. Two such compounds turned out to be potent enzyme inhibitors and to inhibit viral replication as well. (Reprinted with permission from *Chemical & Engineering News*, 7 June 1993, p. 28. Copyright (1993) American Chemical Society)

AIDS virus detected in single cells

Researchers have used *in situ* polymerase chain reaction (PCR) amplification of viral genetic material coupled with flow cytometry to detect the human immunodeficiency virus (HIV-1), which causes AIDS, in individual blood cells. Steven M. Wolinsky and co-workers at Northwestern University Medical School, Chicago, used PCR to amplify HIV-1 proviral DNA - the DNA copy of the viral RNA genome that is integrated into the infected individual's DNA - and HIV-1 messenger RNA sequences inside infected blood cells. The researchers then hybridized the amplified DNA and mRNA inside the cells with complementary fluorescein-labelled oligonucleotide probes. They used flow cytometry, which can sort cells that fluoresce from those that do not, to detect cells infected with HIV-1. The researchers discovered that in HIV-1-infected patients, 4-15 per cent of peripheral blood lymphocytes were infected with HIV-1. The percentage of these cells that contained HIV-1 mRNA, an indicator of viral replication, ranged from less than 1 to 8 per cent. The data indicate that, in HIV-1-positive individuals, a significant proportion of peripheral blood lymphocytes are infected with HIV-1, but that the virus is in a latent state in the majority of these cells. (Reprinted with permission from *Chemical & Engineering News*, 17 May 1993, p. 31. Copyright (1993) American Chemical Society)

Injection of HIV genes elicits immune response

Mice inoculated with genes from the human immunodeficiency virus-1 (HIV-1) develop humoral and cellular immune responses that would be desirable in a vaccine to prevent AIDS. David B. Weiner, of the University of Pennsylvania School of Medicine and the Wistar Institute, Philadelphia, led a team of researchers who injected mice with a plasmid that contained the genes for the HIV-1 envelope glycoprotein gp160 and the HIV-1 Tat and Rev proteins. They found that the mice produced antibodies that neutralized HIV-1 infection and inhibited HIV-1-mediated fusion of lymphocytes, a hallmark of HIV-1 infection. The mice also produced cytotoxic T cells specific for gp160-expressing target cells, indicating that the cellular arm of the immune system also was activated by the inoculation. The researchers say that the approach "which combines the positive aspects of immune stimulation inherent in live attenuated vaccines with the safety of recombinant subunit vaccines, could have wide application in humans and animals". (Reprinted with permission from *Chemical & Engineering News*, 3 May 1993, p. 42. Copyright (1993) American Chemical Society)

Research on bacterial genes

Enzyme gene cloning of methanol-assimilating bacteria

A research group has cloned three genes for enzymes that participate in the biosynthesis of polyester in the methanol-assimilating bacteria *Paracoccus denitrificans*. At present, the group plans to continue their analysis with the goal of improving the productivity and the efficiency of polyester production by this strain. Polyester produced by microbes has attracted attention as a raw material for biodegradable plastic, and various studies have been carried out, but there are almost no studies using methanol-assimilating bacteria.

The research group had already discovered that in a nitrogen-deficient environment *P. denitrificans* synthesizes the polyester copolymer P(3HB-co-3HV) from 3-hydroxybutyric acid (3HB) and 3-hydroxyvaleric acid (3HV) using methanol and n-amyl alcohol as carbon sources, and they conducted research on the production of polyester by this strain. At the same time, they incorporated genetic research to gain a basic understanding of polyester biosynthesis, and now they have succeeded in making the first clones of genes for enzymes that participate in polyester biosynthesis.

With the goal of isolating the genes for polyester synthesis, the group did a Southern hybridization using as probes *phbA* (the β-ketothiolase gene), *phbB* (the acetoacetyl-CoA reductase gene), *phbC* (the PHB polymerase gene), etc., from the polyester-synthesizing bacteria *Alcaligenes eutrophus*, whose base sequences have already been determined. Results gave bands at 1.1 kb for *phbC* and bands at 6.5 kb for both *phbA* and *phbB*, which confirmed gene homology between *P. denitrificans* and *A. eutrophus*. Using a DNA gene bank for this same strain, they performed colony hybridization for each gene and succeeded in cloning the genes for the enzymes β-ketothiolase, acetoacetyl-CoA reductase, and PHB polymerase. Currently the group is making a detailed analysis of each DNA fragment, which they hope will

contribute to more efficient production by this strain as their research progresses.

Along with chemically synthesized compounds and products from nature, polymers synthesized by microbes are being studied and developed as raw materials for biodegradable plastic, but little research has been conducted on methanol-assimilating bacteria. (Source: *Kagaku Kogyo Nippo*, 9 April 1993)

Ajinomoto produces transglutaminase using *E. coli*

Ajinomoto has succeeded in expressing transglutaminase in *E. coli* bacteria. The enzyme can function to link together proteins that make up food. Quantities produced at present are extremely small, but improvements could make mass production possible. Genetic manipulation changing the properties of the enzyme also appears possible. Ajinomoto and Amano Pharmaceutical have jointly developed the microbe-derived transglutaminase. Amano Pharmaceutical is handling manufacturing, and Ajinomoto is marketing the enzyme for use in food processing. Once a genetically recombinant form is placed on track, possibilities will exist for applications beyond food processing, such as pharmaceutical adjuvants and fine chemicals.

As an enzyme able to form cross-links between glutamin and lysine residues, amino acids that make up proteins, transglutaminase is effective in improving food quality: for example, in terms of firmness and flexibility. Previously, only an animal-derived version taken from guinea pig livers existed, but Ajinomoto and Amano Pharmaceutical discovered a transglutaminase-producing actinomycin. Through a fermentation process, Amano Pharmaceutical now manufactures the enzyme for use in food processing, and Ajinomoto has pursued marketing since April.

In order to broaden the activity of transglutaminase, Ajinomoto attempted production using *E. coli* bacteria. Transglutinamase genes were first chemically synthesized and then incorporated in *E. coli* bacteria. The transglutaminase produced accumulated in the periplasm, located between the outer cell membranes.

The amount produced was extremely small. Speculation over the small quantity is that the enzyme breaks down immediately after formation. If the breakdown can be explained, mass production may be possible.

There are no differences in the activity of animal- and microbe-derived transglutaminase, but differences do exist in molecular weight, optimal temperature, temperature stability, and pH stability and the like. Genetic manipulation changing the properties of the microbe-derived transglutaminase also appears possible. (Source: *Nikkan Kogyo Shimbun*, 7 June 1993)

Elucidation of amino acid sequencing

Professor Yasuro Oshimo of the Life Sciences Department, Tokyo Institute of Technology, has developed a method for determining the amino acid sequence important for increasing heat resistance of enzymes. In this method the researcher cultures recombinant bacteria in a special medium that contains a mutagenic substance, studies the enzymes made by mutant bacteria that exhibit increased heat resistance, and determines which amino acid sequence holds the key to heat resistance. This method is expected to aid research on heat-resistant enzymes used in diagnostic drugs, foods and detergents.

Professor Oshima deleted the gene for the enzyme that synthesizes leucine (one of the amino acids) from a certain strain of bacteria and replaced it with the leucine-synthesis gene taken from a strain of *E. coli* that dies at temperatures above 45° C. He then placed this recombinant bacteria in a leucine-deficient medium and incubated them at 80° C. In this medium, the bacteria try to make leucine in order to survive, but the temperature is so high that the enzyme loses its activity, and the bacteria die from leucine deficiency.

If a mutagenic substance is present during culturing, however, mutant bacteria that manufacture a heat-resistant form of the enzyme will survive. If this heat-resistant enzyme is then extracted and compared to the original enzyme, the amino acid sequence that confers heat resistance can be clearly identified. For improving enzyme heat resistance on a practical level, it will just be necessary to target this amino acid sequence and redesign the enzyme by making additional substitutions with different amino acids.

It is possible to use a computer to predict which amino acids are important in improving the heat resistance of an enzyme, but the accuracy is poor. Further, with traditional methods for seeking out mutant bacteria from bacteria in general, it is difficult to eliminate mutants other than the desired ones such as those that make many unrelated substances or, conversely, those with weakened heat resistance. Therefore, isolation and selection has been a tedious process, but this method has the advantage that only heat-resistant bacteria survive. (Source: *Nikkan Kogyo Shimbun*, 3 June 1993)

Bacteria hold the key to genetic pollution

Experiments off the coast of Florida have provided the first evidence that marine bacteria take up DNA that is floating freely in seawater. The bacteria also dismantle the DNA they receive, reassembling it in a different order.

The findings may reinforce fears that when biotechnologists release genetically-modified organisms (GMOs) into the environment, the "foreign" DNA they carry will find its way into native species, a process called

transformation. But on the other hand, the results suggest that nature can absorb fresh genetic material with ease, and without any harmful consequences, so advocates of gene technology may be reassured by the findings, say the researchers.

Governments around the world have introduced legal and voluntary safeguards to minimize the risks of "genetic pollution" when GMOs are released. Opponents fear that the safeguards will not be strict enough. They worry, for example, that DNA conferring resistance to pesticides will spread to weeds, or that genes conferring resistance to antibiotics will spread to harmful bacteria with unforeseen consequences.

Since 1990 Marc Frischer and his colleagues at the University of Florida have been trying to find out how bacteria swap DNA in their natural environment. Their latest research suggests that up to 10 per cent of the marine bacteria in a population are capable of absorbing DNA floating around in tiny loops called plasmids.

Frischer says that all the conditions in seawater are right for natural transformation to occur. Even when bacteria die, their DNA can be inherited by their living relatives.

Knowledge of how bacterial populations move DNA around could be extremely useful in the development of strains that destroy pollutants, for example.

Frischer's insights come mainly from experiments with harmless *Vibrio* bacteria related to *Vibrio cholerae*, the strain that causes cholera. Frischer and colleagues discovered a "high frequency of transformation" (HFT) strain of *Vibrio* called (HFT) *Vibrio* WJT-1C.

In recent experiments, Frischer showed that (HFT) *Vibrio* WJT-1C could accept free-floating plasmids in a wide range of natural conditions typical of tropical and subtropical estuaries. It occurred in the conditions of saltiness typical of estuaries and over the range of temperatures expected (4° C to 33° C).

In subsequent research, Frischer and colleagues claim to have confirmed that natural organisms other than HFT *Vibrios* are capable of absorbing plasmids.

This finding - one which has been observed by other groups - showed that the recipient bacteria reshuffled the DNA. "When you cut up a plasmid, you expect to get a kind of 'map'", explains Frischer. This map indicated subregions of DNA within the plasmid which have different functions, such as the gene itself, and special regions that promote or suppress activation of gene expression. "In transformants, the map had changed", says Frischer. From their data, Frischer and colleagues have estimated the amount of transformation that occurs naturally in the environment. (Source: *New Scientist*, 7 August 1993)

The secret of Bath's methane-fuelled microbes

A bacterium that lives in hot springs at Bath (UK) obtains its carbon and energy by turning methane (CH_4) into methanol (CH_3OH). Until now, the secret of how *Methylococcus capulatus* (Bath) does this at just 45° C, and in a single step, has been elusive. But now chemists in the United States believe they have discovered the mechanism.

Teams headed by Martin Newcomb at Wayne State University, Michigan, and Stephen Lippard at the Massachusetts Institute of Technology, focused on the enzyme responsible for the reaction - methane monooxygenase. They fed *M. capulatus* a "mechanistic probe" - a molecule that the bacterium could digest and so turn into compounds that might reveal how the enzyme's active site works. The chemists used as their probe cylopropanes - cyclic hydrocarbons made of three carbon atoms arranged in a ring. The cyclopropane that they found most useful was called *trans*-2-phenyl-methylcyclopropane.

Newcomb and his colleagues discovered that the enzyme works not by turning methane into a free radical, as they expected, but by boosting its own oxidizing power. The bacteria from the hot springs use water-soluble methane monooxygenase to bring about the key reaction: $CH_4 + O_2 + 2H^+ + 2e^- \rightarrow CH_3OH + H_2O$. The electrons ($e^-$) and hydrogen ions ($H^+$) come from the reduced form of the coenzyme nicotinamide adenine dinucleotide.

Methane monooxygenase is a protein made of three components: a hydroxylase enzyme which binds the reactants methane and oxygen; a reductase enzyme that provides the electrons; and a "coupling" protein that controls the transfer of electrons between the two. The reductase component contains a cluster of two iron and two sulphur atoms, Fe_2S_2, in which the iron is in the reduced state iron (II).

Newcomb and his colleagues found that methane monooxygenase converts the *trans*-2-phenlymethyl-cyclopropane into two products. In one, the methyl group has an OH added, and is in effect a derivative of methanol. In the other, the OH is attached to the benzene ring.

Newcomb and Lippard found that the two products were formed in equal amounts. They say this is because there are two isomers of *trans*-2-phenylmethylcylopropane - a left- and a right-handed form. The two isomers fit into the enzyme's active site in different ways, causing the OH group to join different parts of the molecule.

The chemists expected to observe the product in which the cyclopropane ring had opened, because this would occur if the mechanism went through a free radical stage. In *Pseudomonas oleovorans*, another bacterium that consumes methane, there is evidence that a free radical mechanism is involved. So it was expected that methane monooxygenase should work in a similar way, by plucking

a hydrogen atom off the methyl group of *trans*-2-phenylmethylcyclopropane to leave a free radical. This would stabilize itself by molecular rearrangement, in which the ring would open and a lone electron would join to the carbon carrying the benzene ring. The addition of the OH would occur at this carbon.

However, the chemists detected none of this ring-opened material in the product mixture, showing that formation of a free radical was not the first step. This was confirmed when the chemists used *trans*-2-phenylmethylcyclopropane in which they had replaced the hydrogens of the methyl group with the heavier isotope deuterium. They fed this to the enzyme and showed that breaking a C-H bond was not the first step.

Newcomb and Lippard propose that methane monooxygenase works by picking up a molecule of oxygen (O_2) from the air and using it to oxidize an iron atom first to iron (III) and then to iron (IV) at the same time splitting the O_2 and placing one of its atoms on the iron (IV). This Fe = O unit is then able to oxidize methane to methanol in a single step, and does the same with *trans*-2-phenylmethylcyclopropane. (Source: *New Scientist*, 20 February 1993)

General

Inorganic double helix is chiral and strongly paramagnetic

An inorganic double-helical structure that self-assembles by hydrothermal synthesis from simple inorganic precursors may have potential as a medium for shape-selective absorption or catalysis. The vanadium phosphate structure was synthesized and characterized by Victoria Soghomonian and Robert C. Haushalter at NEC Research Institute, Princeton, NJ, Qin Chen and Jon Zubieta at Syracuse University, and Charles J. O'Connor at the University of New Orleans. The material contains vanadium oxo pentamer units bonded together by P^{5+} cations to form chiral double helices. The helices are entwined so as to form tunnels and cavities that are filled with $(CH_3)_2NH_2^+$ and K^+ ions (derived from reagents used in the synthesis). The tunnels and cavities, along with the material's chirality, suggest potential applications as a shape-selective absorption medium or as a catalyst that might discriminate between enantiomers. The material is also strongly paramagnetic at high temperatures. "Since the material is chiral and magnetic", says Haushalter, "it might provide an opportunity to study the relationship between the rotation of polarized light and an applied external magnetic field." He says the work also helps point the way toward an ability to make inorganic structures that rival organic and biological structures in complexity. (Reprinted with permission from *Chemical & Engineering News*, 15 March 1993, p. 18. Copyright (1993) American Chemical Society)

DNA that binds thrombin exhibits new structural motif

DNA "aptamers" that bind and inhibit the enzyme thrombin have been found to have a structure that indicates they could possibly inhibit other enzymes as well. Aptamers, oligonucleotides that bind specific molecular targets, are isolated by *in vitro* selection (or directed evolution) - the repetitive screening of large pools of randomized molecules for those that bind best to a target. In 1992, Louis C. Bock, John J. Toole, and co-workers at Gilead Sciences, Foster City, CA, isolated aptamers that interact strongly with thrombin, a key blood-coagulation enzyme and a major focus of anticoagulant therapy. Ke Yu Wang and Philip H. Bolton of Wesleyan University, Middletown, CT, working with Gilead scientists, have now determined the three-dimensional structure of the consensus sequence of the most strongly binding of these aptamers. The structure is one that has never been seen before, but it bears an unexpected resemblance to telomere DNA (DNA located at the ends of eukaryotic chromosomes). "This remarkable coincidence", say the researchers, "suggests that this aptamer may have interesting interactions with the proteins responsible for synthesizing and stabilizing telomere structure and that this structural motif may be useful in inhibiting enzymes other than thrombin." (Reprinted with permission from *Chemical & Engineering News*, 15 March 1993, p. 18. Copyright (1993) American Chemical Society)

What makes an animal?

Compared with a plant or a fungus, all animals are pretty similar: if not all equal, at least more equal to each other than to anything else. They all have regular body plans with set numbers of limbs, eyes, vertebrae or what have you laid out in an orderly way.

The body plan is a pattern in time as well as space - the result of the fixed unfoldings of embryonic development. The mechanisms of this process provide biology with one of its most profound problems, which is also one of its most intractable. Over the past 10 years the genetic mechanisms involved have turned out to be almost universal. The genes that do the job in one species of animal can make a reasonable fist of it in another, even if the species are as different as flies and mice. These versatile genes fashion bodies from cells as the master-masons of the Middle Ages built cathedrals from well-hewn stones. And like the masons, the symbol of their guild is a tool. For the genes it is the homeobox.

Homeoboxes are found in organisms as diverse as yeast, tobacco and chickens, which shared their last common ancestor 2 billion years ago. They are sequences of DNA which, when a cell reads the genes and makes proteins from them, produce an almost identical little twist in the structure of the protein, be it in fungus or fowl.

In 1992 Walter Gehring and Kurt Wuthrich, of the University of Basle, showed that the tool is in fact a wedge. The twist fits snugly into the grooves of a DNA double helix and sticks there. Thus anchored, the rest of the protein can perform its allotted task. Any gene that contains a homeobox is thus going to be a gene that controls other genes.

There are various other such DNA-binding shapes to be found in genes for controlling DNA. The interesting thing about homeoboxes is that, in animals, the genes which carry them often come in clusters, laid out along a single stretch of DNA. Not only do they live together, they work together. Each cluster forms a team; the team members tell the cell they are in where it is in the body, and thus what to become.

Geneticists studying body plans have favoured the fruit fly; it breeds fast and keeps its genes on simple chromosomes.

When a fly embryo is four hours old, a gene called *Engrailed* gets switched on. It helps the body divide up into segments. Each segment then develops independently, under the control of homeotic genes found in two neighbouring clusters. *Antennapedia*, for instance, is normally turned on only in the cells that make up the thorax.

Most homeotic genes work in combination. The more of the three genes in the *Bithorax* cluster that are active in a cell, the further it is from the animal's head. The order in which the genes are turned on matches the order in which they are written on the DNA. Head genes are at the front of the cluster and are switched on first. Tail genes are at the end.

It was in the sequences of these genes that, 10 years ago, William McGinnis, currently at Yale University, and Matthew Scott, now at Stanford, first spotted the homeoboxes that they all share. The discovery showed that the genes share form as well as function, using their wedges to locate their genetic targets and thus beginning the process that turns cells-of-all trades into the meat, nerve and sinew of particular organs.

In 1986, Alexander Awgulewitsch, currently at the Medical University of South Carolina, found that clusters of homeobox genes in mice do something similar. These are known as *Hox*, followed by a letter and a number. So far, 38 clustered *Hox* genes have turned up in mice. And they are recognizably the same genes as those in flies; their genetic lettering is often similar even in the bits outside the homeobox, they are activated in the same order during embryonic development, and they seem to do similar jobs.

There are differences, of course. Mice (and other vertebrates) have four *Hox* clusters, each laid out along a different chromosome. Recent work has shown that in vertebrates one entire *Hox* complex is given over to limb development.

Homeobox clusters are now being discovered everywhere, from frogs to flatworms. Some biologists, such as Jonathan Slack and Peter Holland at Oxford University, are beginning to think of them as fundamental. Rather as chlorophyll, which allows photosynthesis, is the defining characteristic of plants, so homeobox clusters, which allow highly structured regular development, may be the defining characteristic of animals.

For over 3 billion years, life on Earth was without multicellular animals, but there were no big animals until the beginning of the Cambrian period. Then, suddenly, the rocks became littered with weird and wonderful fossils, evidence of a massive experiment with new body plans. In a shockingly brief time - new evidence from Siberia puts it at a mere 5-10 million years - all the basic designs seen in today's animals appeared, along with a vast number of other designs since discontinued. Segmentation appeared, and with it the possibility of getting bigger by adding segments. And almost all the new animals had length: unlike jellyfish, they had heads and tails.

It is known as the Cambrian explosion: to palaeontologists, who have a bias towards animals, it is surpassed in mystery and import only by the origin of life itself. Some scientists favour a change in the physical environment as an explanation, and point to increases in the amount of oxygen around and changes in the composition of seawater (which might make shells possible). Others prefer biological causes: "loose niches" for the ecologically minded, "loose genes" for the genetically inclined.

There are two reasons for preferring biological explanations. The explosion was, in geological terms, breathtakingly fast; and it was restricted to animals. There is no evidence of parallel increases in the number of, for example, plants. So David Jacobs of the American Museum of Natural History suggests that the cause may have been a combination of the over-eager replication of one homeobox gene, which turned itself into a cluster, and the arrival of segmentation. The result was a way of telling cells to do slightly different things. If so, it was a fortuitous change. When you are bigger and more sophisticated than anything else around, you can afford to experiment. You may even take over the world. (Source: *The Economist*, 18 September 1993)

Fullerene bioactivity

A water-soluble derivative of buckminsterfullerene is active against HIV-1 and HIV-2, the human immuno-deficiency viruses that cause AIDS, according to work by two independent research groups. The derivative is not a drug candidate itself, but it offers a potential lead for designing anti-HIV agents, say scientists in the two teams. This is the first example found of biological activity by fullerenes.

Researchers at the University of California, San Francisco, noted that C_{60} has dimensions and properties

that suggest it might block the active site of a key viral enzyme, HIV-1 protease. Scientists at Emory University in Atlanta recognized that a suitably derivatized C_{60} would share some properties with a class of large inorganic clusters that inhibit another HIV enzyme, reverse transcriptase - and, as such, might also inhibit the enzyme.

Both groups turned to chemistry Professor Fred Wudl of the University of California, Santa Barbara, to create the water-soluble fullerene derivative they used. Fullerenes are very hydrophobic and essentially insoluble in aqueous media. Wudl and co-workers developed a facile technique for selectively functionalizing fullerenes.

The UCSF discovery represents an unlikely intersection of three areas of intense research activity: fullerene chemistry, development of HIV-1 protease inhibitors, and rational drug design. (Abstracted with permission from *Chemical & Engineering News*, 2 August 1993, p. 3. Copyright (1993) American Chemical Society)

Searching for DNA sequences in a flash

The race to make sense of data from the human genome could be speeded up significantly by a computer technique borrowed from the field of image processing. Its developers at IBM's research centre in New York State say that it now takes seconds instead of hours to search for meaningful patterns in DNA.

Computers can help to pick out the patterns of exons. In the same way the sequences of amino acids in proteins need to be identified. Identical or similar sequences of exons and amino acids in different species can reveal evolutionary relationships.

Exons can only be spotted at present by comparing them with known exons, or by spotting "marker" sequences that signal their beginning and end. Searching is made more difficult because exons can be interrupted anywhere by introns. Amino acid sequences, however, are simpler and not interrupted.

Isidore Rigoutsos and Andrea Califano, of the computational biology and pattern matching group at IBM's Thomas J. Watson Research Center, compare the search for both types of sequence to looking for a phrase in a shelf of books. The conventional computational approach is to read through all the books. But it would be quicker if the books were indexed according to their contents, as is done in all libraries, so the search could begin with the most likely books. Shelves marked "colours", "brown", "mammals" and "foxes" could all have notes saying "see book 8, page 9, paragraph 3", for example. This is what the new algorithm, the Fast Lookup Algorithm for Sequence Homology (Flash), does with the DNA sequences.

Known sequences are indexed in as many different ways as possible. A sequence submitted for comparison is analysed and indexed in the same way, then the "shelves" are searched to find the notes.

Rigoutsos says introns and mutations in sequences are not a problem. If "the brown fox" were a known sequence and "the quick brown fox" were a new one, a search using the index "fox" would throw up "the brown fox". If a sequence has no matches, researchers can investigate it further to see if it carries useful information and should be added to the known sequences.

Genome researchers usually have 100 amino acids or exons to compare with known sequences at a time. Rigoutsos creates indices using not the entire sequence but parts, such as the first, fourth and sixth amino acid. Flash takes about 8 seconds per search, using spare processing capacity on other researchers' RS/6000 workstations. Califano says the alternative approach, of searching systematically through a random list of sequences, takes 20 times longer, even using a parallel processing computer which examines many parts of the list simultaneously.

Flash takes the same time to do a search, regardless of the number of known sequences. That figure is expected to multiply by a factor of 2,000 by the end of the decade, which will make traditional searching methods so slow as to be practically unusable.

IBM's database stores only the indices; the sequences are kept separately. Several thousand newly established sequences are added every four to five months, and indexing them only takes a few hours. About 30,000 amino acid sequences are stored at present, from people, yeasts, nematode worms and other genomes. Their indices take up about 20 gigabytes. Shortly all the known DNA sequences should also be indexed. Researchers who come up with new sequences simply send an electronic mail message to IBM and the possible matches are sent back automatically in a few minutes. Most of this time is taken up by information travelling between the computer and the disks where the indices are stored.

IBM has about 200 people testing Flash, and has calculated that it will find 99 per cent of all sequence similarities in a few seconds.

This class of algorithm was developed to help robots recognize similar, but not identical, objects. IBM is considering applying Flash to text searches and retrieval and speech recognition, which all depend on pattern recognition. It is also using Flash to find the similarity between new and existing molecules, which can speed the search for new drugs. Molecules with similar properties often have completely different shapes and structures, but their "fingerprints" from a spectrometer will be alike. At

present they are compared by hand, or by powerful computers. (Source: *New Scientist*, 7 August 1993)

March is the cruellest month

Schizophrenia affects about one in 100, and it is fatal in about 10 per cent of cases when the sufferer commits suicide. Environmentalists argue that it is too common to be genetic: if a gene caused it, natural selection would have all but eliminated it years ago. There are links between schizophrenia and social deprivation that environmentalists can look to for support. Yet there is also medical evidence that schizophrenia runs in families. Some studies show that abnormalities in the brain which seem to be associated with the disease are often present early on in life - indeed, at birth. If the environment is having an effect, it is doing so early on.

In the northern hemisphere, people born in March are 15 per cent more likely than average to suffer from schizophrenia: this suggests that something happens in winter. Robin Murray of the Institute of Psychiatry in London and Eadbhard O'Callaghan on the Cluain Mhuire Family Centre in Dublin believe that the something is influenza. Following up a study which showed that the 1957 epidemic of Asian 'flu in Helsinki was associated with a rise in schizophrenia, they correlated the birthdays of 15,000 English schizophrenics with the ferocity of the previous winter's epidemic, as measured by the number of people who died in it. The worse the epidemic, the more schizophrenics turned up.

Dr. Murray and Dr. O'Callaghan do not believe that all schizophrenia is linked to 'flu, or that the virus causes the problem directly. Viruses cannot cross the placenta, and so cannot infect the foetus. Antibodies, however, can get across, and can reach the foetal brain. The barrier that shields adult brains from hostile chemicals is not yet fully developed in a few-months old foetus.

The scientists' best guess is that a protein in the pyramidal cells of the foetal hippocampus (the part of the brain that seems most affected in schizophrenics) looks, to the antibody, similar to one in the virus. That may lead the mother's antibodies to attack the foetal cells. The researchers have evidence to support their idea. Peter Lang of Nottingham University has found that the antibodies rabbits make against 'flu react strongly with protein from the hippocampus.

The nature of the antibody and the hippocampal protein will depend on the genes that describe them - some antibodies or some proteins may be more likely to go in for this sort of self-inflicted damage. If Dr. Murray and Dr. O'Callaghan are right, that may go some way to explaining why schizophrenia persists. (Source: *The Economist*, 28 August 1993)

Women leave indelible mark on evolution

Women, rather than men, are the most important agents of human evolution, say scientists in Israel. They believe recent work on "gene imprinting" has brought the field of genetics as a whole, and human evolution in particular, to the verge of a fundamental revolution.

Imprinted genes are a small but important minority of genes which are "marked" in such a way that identical genes inherited from a person's father and mother function differently. One is suppressed and inactive while the other is fully functional, depending on whether they have come from the paternal or the maternal line. Recently, imprinted genes have been implicated in several genetic disorders and childhood diseases ("Why genes have a gender", *New Scientist*, 22 May).

In placental mammals, imprinted paternal genes are responsible for the development of the placenta, while imprinted maternal genes are involved in the growth of the embryo.

Abraham Hochberg, Nathan de Groot and Jacob Rachmilewitz of the Hebrew University in Jerusalem recently studied the imprinted gene phenomenon in abnormal placentas called "moles".

The imprinted genes received from the father seem to do much of the work in providing the foetus with its organ of nourishment *in utero*. The imprinted genes from the mother, however, play a critical role in the way the foetus, and subsequent baby, develop.

Together with Bernard Gonik of the University of Texas Medical School, the researchers wrote a paper soon to appear in the Canadian journal *Medical Hypotheses*, in which they suggest that the key to human evolution is the preferential expression of maternal imprinted genes.

For human evolution to proceed, explains Hochberg, favourable mutations must be expressed and passed on to the next generation. An imprinted gene inherited from one's father will receive no expression in embryonic or later development, but an imprinted maternal gene will always be expressed, he says.

This pattern of genetic inheritance completely upsets the framework of classical Mendelian genetics, says Hochberg. The discovery of imprinted genes means that

the simple dominant-recessive model, while true for most genes, is unable to explain a small but critical group of them. (Source: *New Scientist*, 24 July 1993)

Commission sees indications that Agent Orange damage is in genes

The New Jersey Agent Orange Commission has found disturbing indications that damage done by the defoliant is being passed on to the next generation.

Two recent studies cited by the National Academy of Sciences in a report on the defoliant led investigators to a conclusion that there is "some suggestion" of an increased risk of cancer in offspring of exposed veterans.

Two other studies used in the report found insufficient evidence to determine if an association exists between the chemical and childhood cancers in veterans' offspring.

The project so far has developed a base of 1,200 children of veterans, and it is hoped to be able to increase that to 18,000 in the next few years.

The New Jersey group has also found that men exposed to Agent Orange have low sperm counts, deformed sperm and impaired immune systems as well as the abnormalities in their offspring 20 years after their exposure to the chemical. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 16 August 1993)

D. APPLICATIONS

Pharmaceutical and medical applications

R&D for second generation remedy for C-type hepatitis under way

The market for interferon has expanded rapidly. In 1992 it reached about ¥ 150 billion (on a drug cost basis), and in 1993 it is expected to approach ¥ 200 billion.

This is because in January 1992 interferon was approved as a drug to treat hepatitis C. Sales of interferon as an anti-cancer drug and as a treatment for hepatitis B were slumping, but this new approval triggered the expansion of the interferon market. The number of patients in Japan infected with hepatitis C is estimated to be 1-2 million. However, treatment with interferon is currently approved only for patients with the severe form of the illness called active chronic hepatitis C. Moreover, it is estimated that only 20-40 per cent of these patients are actually treated with interferon. There is still a strong need for a new "miracle drug" for hepatitis C. With an eye on the post-interferon age, R&D has already begun on a second-generation hepatitis C drug to capture this huge market.

Protease (proteolytic enzyme) inhibitors are attracting attention as the first second-generation drug. It has recently been discovered that in the case of the hepatitis C virus, the first protein to be synthesized in the liver cells is a long string of 3010 amino acids. Then proteases unique to the virus cleave this long protein at specific sites to form eight or nine smaller proteins that make up the viral coat, core proteins, enzymes, etc.

Scientists speculate that if a drug can be developed to inhibit these proteases, then virus replication will be blocked.

Actually, in the rush to find a drug for AIDS the Swiss company Hoffman-La Roche developed a protease-inhibiting drug for the AIDS virus in 1992 and clinical tests within the EC have already begun. In Japan, Nikko-Kyodo (Nippon Mining Co.-Kyodo Oil Co.) and Kyoto Pharmaceutical University have jointly developed an AIDS virus protease inhibitor. Plans call for clinical tests to begin in the US under the auspices of the National Institutes of Health.

Up until now, the main antiviral drugs used to treat AIDS have been nucleic acid derivatives such as AZT (azidothymidine), but now the focus is shifting to the development of virus protease inhibitors.

Compared with the work on AIDS, we have just begun to scratch the surface in developing a protease inhibitor for the hepatitis C virus. In fact, the proteases themselves have only recently been identified. According to research at the National Cancer Center, the hepatitis C virus uses two types of proteases called Cpro1 and Cpro2.

The proteases can be classified by their chemical properties and the types of amino acids they cleave. Cpro1 is a metal protease, and Cpro2 is a serine protease. The AIDS virus protease is an aspartate protease, so it has different cleavage site specificity.

Large amounts of proteases are present in the normal human body as well. If these enzymes are also inhibited by the drug, there will be unpleasant side effects. Therefore, whether a hepatitis C protease inhibitor can be developed into a hepatitis C therapeutic drug will depend on whether a compound can be found that specifically inhibits only the target proteases of hepatitis C.

Japan's Drug Creation Technology Research Institute, which was jointly established in March 1992 by 20 private companies and the Ministry of Health and Welfare's investment organization, the Foundation for the Study and Relief of Drug Side Effects, has begun screening hepatitis C virus protease inhibitors in cooperation with the National Cancer Center.

They have spliced the Cpro2 gene into a baculovirus and synthesized large amounts of recombinant Cpro2 in insect cells. This research involves adding a large number of substances found in nature such as chemical compounds, micro-organisms, herbs, marine products, etc., to this recombinant protease and checking the enzymatic activity

to try to come up with inhibitors. So behind the scenes many companies are rushing ahead with similar research. (Source: *Nikkei Sangyo Shimbun*, 9 April 1993)

Tumour shrinking immunotherapies

Two companies have reported surprising results - ranging from a 50 per cent tumour shrinkage to total disappearance of malignant cells - in Phase I cancer immunotherapy trials.

If more advanced studies back up the early findings, both firms plan to ask the US Food and Drug Administration (FDA) for a way to move these agents rapidly through the approval process.

Biomira Inc. (Edmonton, Canada) and **Immuno Therapeutics Inc.** (Moorhead, USA), saw tumours regress in several patients in their Phase I trials. Such studies are designed to set dose and gauge safety, but not to demonstrate efficacy.

In the trial, the vaccine - a synthetic carbohydrate antigen linked to a carrier protein - was given to 12 breast cancer patients. These patients had completed, but were not helped by conventional chemotherapy. In the Phase I trials, the vaccine was safe and successfully stimulated antibodies against the tumours.

Two of 12 patients had a partial response. Tumours shrank by more than 50 per cent in those patients. In others, the vaccine stopped the growth and spread of the cancer.

Two patients had mixed responses, meaning that one tumour decreased in size, while others grew.

Biomira has created a totally synthetic vaccine. They start by copying an epitope, known as sialyl-Tn (STn), found tumour surface cells. This antigen, which is not seen in all patients, is associated with a poor prognosis. The company is developing a method of predicting clinical outcome based on the amount of this antigen physicians can find in the malignant tissues.

Biomira started Phase II trials in 1992 in 40 breast, colon and pancreas cancer patients in Canada and another 20 patients in the UK in 1993.

In another reported trial, immunotherapy prompted remissions in colorectal cancer patients with disease that had spread to the liver.

Called ImmTher™, the drug consists of a repeating double sugar isolated from BCG linked to a peptide that activates macrophages, stimulating them to recognize and destroy tumours. Other companies have experimented with a single sugar version, but the double sugar appears to be more active.

Of a total of 13 patients treated, three had tumours in their livers. All three of these patients experienced a regression. Following the end of therapy, the patient lived for 2½ years, about twice the average life-span of late-stage colorectal cancer patients who receive the approved chemotherapy regimen.

ImmunoTherapeutics started Phase II studies, which will ultimately include 30 patients with colorectal cancer that has spread to the liver. Unlike an earlier Phase II trial, this study will be conducted on patients who have received no previous cancer drug treatment. In the earlier Phase II trial on heavily pre-treated patients, the drug stopped the spread of the disease, but there was no shrinkage.

Vosika said that the product works on liver metastases because the liver has a high level of macrophages His company is planning to investigate the immunostimulant in other cancers which usually metastasize to macrophage-rich regions of the body, such as the bone marrow.

Cancer immunotherapy, a concept that is at least 100 years old, has met with a lot of skepticism in the past, because the early immunostimulants were so crude that it was hard to tell what was eliciting the immune response. But recent advancements in the understanding of tumour biology and the immune system is showing scientists how to develop highly sophisticated agents, and the technique is rapidly gaining credibility. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 19 April 1993)

Researchers make new taxol

Scientists at the State University of New York at Stony Brook say they have synthesized a taxol-like compound more powerful than the rare Pacific yew-based anticancer drug. Dr. Iwao Ojima, says the chemical should avoid nerve damage and heart arrhythmia side effects of the original. The chemical is derived from a form of baccatin found in European yews. (Source: *Chemical Week*, 7 April 1993)

Generating new cartilage growth

A new technology being developed by OsteoBiologics Inc. in San Antonio, could have a dramatic impact on millions of people who suffer disease or injury to their knees, hips or other joints each year. The device - a tiny, biodegradable implant - would allow surgeons to go beyond traditional treatments (such as removing damaged cartilage) and encourage regeneration of new cartilage and bone growth. The implant contains bioactive factors that enhance both the migration and development of cartilage and bone cells. Inserted arthroscopically, the implant dissolves over time and is replaced by new cartilage. Because it has the same physical properties as cartilage, the implant would allow the patient to walk soon after surgery and eventually regain use of the knee, hip or other affected

joints. OsteoBiologics is in the Texas Research and Technology Foundation's Technology Incubator at the Texas Research Park. (Source: *BioBytes. San Antonio Biotechnology News & Information*, May 1993)

Future malaria vaccine development

A two-step immunization experiment, using two recombinant vaccines, is redefining malaria vaccine development. For years, it was thought that one vaccine, a single antigen evoking a single immune response, could ward off this often deadly disease.

The malaria parasite undergoes a dramatic metamorphosis in the body, and it has been impossible to come up with one vaccine to catch it.

When malaria bugs are transmitted from mosquito vector into mammalian host, the sporozoites are worm-shaped and evoke an antibody response. But when they move to the liver, they provoke another part of the immune system - CD4 and CD8 T cells. By the time they invade the bloodstream, where they attack red blood cells and secrete blood-poisoning toxins, the sporozoites are shaped like little balls.

In a study, published in the *Proceedings of the National Academy of Sciences* (PNAS) of 1 June 1993, scientists report they believe they may have discovered a strategy to checkmate malaria. While the system has only been tried in mice on the rodent-specific version of the parasite (*Plasmodium yoelii*), the researchers hope the approach will translate into human terms.

The trick, said immunologist Fidel Zavala, of the New York University School of Medicine, one of the study's principal investigators, is in figuring out how to activate multiple arms of the immune system against a target that is as elusive and fast-moving as its insect vector.

Two recombinant vaccines, one an influenza virus carrying a cytotoxic epitope of the malaria antigen and the other a vaccinia virus with the entire protein, were sequentially injected into mice. When challenged, these mice were protected against the disease.

This two-vaccine combination apparently had a synergistic effect. Two shots of influenza virus, or of a vaccinia virus, did not protect the mice. The agents were also ineffective when the order in which they were administered was reversed.

It appears that the influenza vaccine may be priming the anti-parasite CD8 cells, which seem to have a significant impact on conferring immunity, said Zavala. Later, the vaccinia vaccine pushes the CD8 cells into action.

Zavala said that the group's next step is to improve immunostimulating properties of the constructs and start to develop vaccines targeted to the four separate malarial strains that infect humans. This approach could have applications in many diseases where a CD8 response is important. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 June 1993)

Joint venture to develop osteoporosis treatment

Allelix Biopharmaceuticals Inc., Toronto, Canada and Glaxo Canada Inc. have formed a joint venture to pursue the clinical development of parathyroid hormone (PTH) as a treatment for osteoporosis.

Osteoporosis is a degenerative bone disease characterized by a decrease in the rate of new bone formation. Affecting mostly women, this disease increases the risk of bone fractures resulting in significant morbidity which can lead to hospitalization and even death. Recent studies have shown that 45 per cent of women over the age of 50 will suffer an osteoporosis-related fracture during their lifetime.

PTH is a protein that plays an important role in regulating bone mineral metabolism in the body. Laboratory studies with Allelix's PTH have shown that PTH actually rebuilds bone in the order of 5-10 per cent over one year. There is a widely recognized need for a treatment which can actually add back lost bone, since the restoration of lost bone can prevent fractures.

The Allelix-Glaxo joint venture will conduct Phase I and II clinical studies to determine the safety and efficacy of PTH for the treatment of osteoporosis. The programme costs will be shared by the two companies. Allelix will retain PTH supply rights. Glaxo will have the option to undertake world-wide clinical and commercial development at the end of Phase II trials. In such case Allelix will receive royalties and Canadian co-promotion rights. (Source: *News Release*, 3 August 1993)

Cystic fibrosis drug gets go-ahead

A US Food and Drug Administration scientific advisory panel has recommended marketing approval of Genentech (So. San Francisco) biotechnology drug Pulmozyme for treating cystic fibrosis. The recombinant human DNase is aimed at reducing the risk of respiratory tract infections and improving lung function. It acts as a "molecular scissor," cutting DNA in the thick secretions typical of the disease, according to Genentech. While the panel's recommendation is not binding, FDA typically follows such decisions. If approved, the biotech drug would be the first new treatment for cystic fibrosis in 30 years. Cystic fibrosis affects about 30,000 Americans, who have a median survival age of 29 years. Genentech says it will launch the drug as "soon as possible" after it receives FDA approval but cautions that there could be delays in the start-up of its new manufacturing plant. Genentech has also applied for approval of the drug in Canada and Europe. (Source: *Chemical Week*, 18 August 1993)

Study suggests genetic vaccination for AIDS

Researchers at the Wistar Institute and the University of Pennsylvania Medical Center in Philadelphia say they have used genetic vaccination to produce in a live animal an effective immune response to HIV-1. Performed in the laboratory of David B. Weiner, Ph.D., and reported by Drs. Bin Wang, Weiner and their colleagues, the research suggests that the same approach, applied directly in humans, might lead to an effective vaccine for the prevention or treatment of AIDS. The scientists injected plasmids containing gp160 from HIV-1 directly into the muscle tissue of laboratory mice to produce an immune response. Eighty per cent of the mice seroconverted after two immunizations and the antibodies were reactive to multiple epitopes, according to Dr. Weiner. He added that the response generated involves both the production of antibodies that inhibit the AIDS virus from infecting cells, and the stimulation of killer T-cells to destroy virus-infected cells.

Currently, the safety and efficacy of a prototype genetic vaccine are being evaluated in primates by Dr. Weiner and scientists from Apollon, Inc. (Malvern, PA). (Source: *Genetic Engineering News*, 15 May 1993)

Rational drug design

Traditionally, the drug discovery process involved the synthesis of a large number of chemical compounds and evaluating them in animal models that mimicked the disease condition of a human ailment. New molecules were synthesized randomly and their biological activities in animals were determined. Only when a compound was found to have some effect in these animal models, would medicinal chemists try to improve that biological activity by systematic modifications to the chemical structure - thus establishing a structure-activity relationship. Because of the complex nature of the whole animal model used in these tests, it was impossible to predict the activity of a given organic molecule or even to explain why certain molecules were inactive. Many compounds that have been discarded in the past, based on whole animal testing, might have had intrinsic biological activity, but were found inactive due, for example, to inadequate distribution of the drug in the target organs.

With the advances made in biological sciences, especially in biochemical pharmacology and molecular biology, diseases could be correlated with certain biochemical measures. Enzymes were discovered that form an integral part of a biochemical cascade, disruption of which may lead to disease conditions. It has long been known that numerous chemicals and peptides are produced in the body that maintain physiological function. These chemicals, called ligands, interact with certain proteins called "receptors" located on the surface of target cells. Thus, essential physiological actions are induced when these ligands interact with their receptors. A drug can also interact with a specific receptor, and thus interrupt the interaction with the body's natural ligand. This process brings about inhibition of the physiological activity normally produced by this particular ligand. Similarly a drug many also interact with a receptor and augment the action of the natural ligand.

This understanding of the ligand-receptor interaction has become of prime importance in therapeutic research. Receptors are membrane bound complex protein molecules, distributed in various organs. A receptor can have several subtypes, activated by the same natural ligand, but located in different tissues of the body to control different physiological functions. Because these different receptor subtypes have different structures, it is possible to design drugs which interact only with particular subtypes. Therefore such drugs will selectively interfere only with the particular physiological processes associated with this receptor.

Drug discovery today depends upon the design of molecules that will interact selectively with a particular receptor subtype that is associated with a particular disease. These organic molecules, because of their selectivity, should have less side effects.

Molecular biology has made a very strong impact on the drug discovery process. Many receptors of human origin have been cloned and produced in sufficient quantities with which to conduct extensive research. By studying the binding of ligands to the cloned human receptors, chemists can design compounds which bind more strongly and selectively with the receptor. Only when compounds are found which have strong binding affinity, are they then tested in animal models. This eliminates unnecessary testing of large numbers of chemical molecules in expensive animal experiments.

Molecular biology can also shed light on the nature of the interaction between the binding site on the receptor and the ligand or drug. This is done by making small changes in the structure of the receptor and examining the effect on the binding interaction. This knowledge, coupled with computer-based molecular modelling studies of the receptor and the receptor-ligand interaction, provides very useful clues to medicinal chemists in designing more effective novel chemical entities.

This is an interactive process where chemistry, molecular biology and biochemical pharmacology play important roles, and has made the drug discovery process less time-consuming. Medicinal chemists need to synthesize fewer compounds in order to achieve the goal of producing effective drug candidates, thus rendering the whole discovery process more efficient and economical. (Source: *Spotlight*, March 1993)

Ruthenium complex active against Chagas' disease

Researchers have developed a potential new drug for Chagas' disease in which the antiparasitic agent clotrimazole is complexed with ruthenium. Chagas' disease, which occurs primarily in Latin America, is caused by a trypanosome parasite and is characterized by fever, oedema, heart attacks, and enlargement of the spleen, liver, and lymph nodes. Some 20 million people in Latin America are believed to have the disease. Nitrofuran and nitroimidazole compounds can be used to treat it, but they have limited effectiveness and cause toxic side effects. Sterol biosynthesis inhibitors like ketoconazole are among the most effective anti-Chagas' agents available, but are also toxic at the high doses required. Now, chemistry professors Roberto A. Sánchez-Delgado and Julio A. Urbina of the Venezuelan Institute of Scientific Research, Caracas, and co-workers have found a ruthenium complex of clotrimazole that is about as strong as ketoconazole, but may be less toxic. In-vitro tests show no toxicity, and tests in mice are under way. The researchers believe the compound, or an analog, could prove to be a safe, effective anti-Chagas' drug, and that similar transition-metal complexes could show activity against other parasitic and fungal diseases such as malaria. (Reprinted with permission from *Chemical & Engineering News*, 2 August 1993, p. 20. Copyright (1993) American Chemical Society)

Synthetic peptides block myasthenia gravis mechanism

In a study that suggests a route to vaccines against myasthenia gravis, researchers have found synthetic peptides that block the proliferation of T-cells involved in the disease. There is currently no cure for myasthenia gravis, an autoimmune disease that causes chronic fatigue and loss of muscle control. In the disease process, receptor proteins at nerve-muscle junctions are broken down and the fragments presented to T-cells, which proliferate in response. This induces the production of specific antibodies that bind to the receptor protein, blocking the nerve-muscle signaling pathway and causing the disease's symptoms. Researchers have now demonstrated that synthetic peptides - each differing by a single amino acid from key fragments of the receptor protein - suppress the pathological proliferation of T-cells, both in cell culture and in live mice. Hence, the analogs could potentially be good candidates for immunomodulatory therapy of myasthenia gravis, they say. The work was done by Yael Katz-Levy, Susan L. Kirshner, Michael Sela, and Edna Mozes of the department of chemical immunology at Weizmann Institute of Science, Rehovot, Israel. (Reprinted with permission from *Chemical & Engineering News*, 2 August 1993, p.20. Copyright (1993) American Chemical Society)

Drug prevents, reverses multiple sclerosis-like disease

In animal studies, researchers have shown that the immunoregulatory drug Linomide (quinoline-3-carboxamide) prevents and reverses experimental autoimmune encephalomyelitis (EAE), a disease that resembles human multiple sclerosis (MS). Various experimental immunoregulatory agents have been tested as anti-MS drugs in recent years, but these often have toxic side effects and efficacy has not yet been demonstrated. In the tests on Linomide, none of 17 mice treated with the drug after induction of EAE developed any symptoms, whereas 19 of 20 untreated controls became severely paralyzed and exhibited the disease's characteristic neural demyelination. Linomide treatment also made mice resistant to subsequent disease induction, and inhibited relapses in animals already paralyzed with the disease. Oded Abramsky and co-workers at Hadas-Hebrew University Hospital, Jerusalem, who performed the tests, say the results indicate that Linomide could be a potential treatment for MS. But the National MS Society, in New York City, cautions that although the animal test results are intriguing, many drugs shown to work against EAE have proved to be ineffective against MS. (Reprinted with permission from *Chemical & Engineering News*, 19 July 1993, p. 32. Copyright (1993) American Chemical Society)

Thalidomide blocks virus that causes AIDS

Thalidomide, the tranquilizer associated with birth defects in the 1960s, blocks activation of HIV-1 - the virus that causes AIDS - in certain immune system cells, find researchers at Rockefeller University in New York City.

The drug could be useful in delaying the progression of an HIV-1 infection and onset of AIDS symptoms, the researchers suggest. They now plan clinical trials to test this idea.

Thalidomide was withdrawn from the market in Europe in 1961 after the discovery that it causes limb deformities in children of women who took it. Since then, researchers have found that thalidomide is useful in treating inflammation associated with severe cases of leprosy. There is evidence that the drug could also be useful in treating other inflammatory processes.

While investigating the mechanism of thalidomide's anti-inflammatory activity, Gilla Kaplan, an associate professor at Rockefeller, and co-workers showed that the drug blocks biosynthesis of tumour necrosis factor-α (TNF-α), a cytokine that can induce the fever, chills, aches, and inflammation associated with infections. TNF-α is also known to be involved in activation of HIV-1 in latently infected immune system cells.

The researchers have shown that thalidomide significantly reduces HIV-1 replication in cell lines latently infected with HIV-1, and in peripheral blood mononuclear cells (PTMCs) from individuals infected with HIV-1.

Although TNF-α also is involved in HIV-1 activation in T lymphocytes, Kaplan's research indicates that thalidomide has little effect on the production of TNF-α by these cells or HIV-1 replication in them

According to Kaplan, thalidomide appears to exert its effect by selectively accelerating the decay of TNF-α messenger RNA in PBMCs. In addition to suppressing HIV-1 replication, thalidomide could enhance the well-being of HIV-1-infected patients by reducing TNF-α-induced fever, malaise, muscle weakness and wasting, Kaplan says.

The researchers have given thalidomide to a number of tuberculosis patients, some of whom were infected with HIV-1. Although there have been some positive signs in these patients, Kaplan says the results are too preliminary to draw conclusions about the effectiveness of the treatment. (Abstracted with permission from *Chemical & Engineering News*, 12 July 1993, p. 5. Copyright (1993) American Chemical Society)

Immunoconjugate fights cancer via Trojan horse approach

An immunoconjugate that uses a Trojan horse strategy to deliver an anti-cancer drug to cancer cells has been found to be highly effective in animal tests. The use of immunoconjugates to fight cancer has been a long-sought goal, but results up to now have been disappointing, with most conjugates showing only about the same efficacy as the unconjugated drugs. Now, immunologists Pamela A. Trail and Karl-Erik Hellström and their co-workers at Bristol-Myers Squibb Pharmaceutical Research Institute in Princeton, N.J., Wallingford, Conn., and Seattle, have done better with a conjugate of the anti-cancer drug doxorubicin and a monoclonal antibody. The antibody binds an antigen found on many human cancer cells, after which it is rapidly internalized into acidic cell organelles. The unique feature of the conjugate is that the drug is released only after internalization, when a hydrazone bond between antibody and drug is cleaved in the acidic environment. The conjugate causes complete regressions and cures of grafted human lung, breast, and colon cancers growing subcutaneously in athymic mice (mice whose immune systems are compromised to prevent tumor rejection), whereas doxorubicin only slows cancer progression or is ineffective. The conjugate also cures most athymic mice with extensively metastasized human lung cancer, and nearly all athymic rats with subcutaneous human lung cancer. Although studies on immuno-compromised animals are not ideal for predicting anti-cancer activity in humans, the findings warrant clinical studies, the researchers say. (Reprinted with permission from *Chemical & Engineering News*, 12 July 1993, p. 24. Copyright (1993) American Chemical Society)

Anti-tumour agents found in cashew apple juice

Researchers at the University of California, Berkeley, have discovered anti-tumour activity in the juice of the cashew apple. Although the cashew nut is still the major commercial product of the cashew tree, the pear-shaped apple to which the nut is attached is available in far greater tonnage and is of growing importance as a food product. Several processes have been developed for converting cashew apples into jams, syrups, and beverages. As part of a continuing effort to screen tropical fruits and vegetables for new anti-tumour agents, Isao Kubo and co-workers in the Division of Entomology & Parasitology at UC Berkeley have found that cashew apple juice has significant *in-vitro* cytotoxicity against breast cancer cells. Several compounds from the juice that exhibit high activity against breast cancer are also about equally cytotoxic to cervical cancer cells. The cytotoxicity of the juice components is not high enough to warrant their consideration as "lead" compounds for drug design. But the data suggest that consuming the cashew apple or its products for long periods of time might have some effect in controlling tumours, the researchers say. (Reprinted with permission from *Chemical & Engineering News*, 28 June 1993, p. 24. Copyright (1993) American Chemical Society)

Genetic "switch" to target skin cancer

A novel approach to gene therapy for melanoma has been developed by British scientists. Early trials of the method could start this year on people with this aggressive form of skin cancer whose existing treatments have failed.

The key to the new method, developed by researchers at the Imperial Cancer Research Fund, is that it exploits a genetic "switch" that flicks on only in the type of cell affected by melanoma. By hooking up potentially therapeutic genes to this switch, the researchers hope to target treatments precisely at the melanoma and leave healthy surrounding tissue alone.

Ian Hart and Richard Vile, scientists at ICRF's London laboratories, warn that their work is at a very early stage and gene therapy for melanoma may not be widely available for another 10 years.

The skin cells that turn cancerous in melanoma are called melanocytes. They make melanin, the pigment that protects against the Sun's rays, with the aid of a key enzyme, tyrosinase. Although the gene for tyrosinase is present in all cells, it is switched on only in melanocytes - by a "switch" gene. In other cells the switch is off. The team reasoned that they might be able to use the switch gene to activate not just tyrosinase, but a range of other genes too - for example, genes that would stimulate a stronger immune response to cancer cells. As long as such genes were attached to the switch, they would act only in melanocytes. In future, other switches could be identified in other types of tumour cell.

Using a "marker" gene, the team tested the idea in mice. Hart and Vile hooked up the switch gene to the marker gene and injected the DNA directly into two types of mouse tumour. In melanoma tumours, the marker gene was switched on. In other tumours containing no melanocytes, the marker gene remained inactive. Healthy surrounding tissue was also unaffected.

The next step was to see if useful genes could be switched on by the same method. The first to be

tested was the gene for interleukin-2, a messenger protein made by cells of the immune system. If melanoma cells can be made to produce IL-2, this should stimulate a strong, specific immune response against them. IL-2 has unpleasant side effects if given in large doses, but targeting it at melanoma cells should be less toxic.

In mice, the team has succeeded in making the IL-2 gene switch on in melanoma cells. They are now waiting to see whether the animals' immune systems destroy the cells.

Doctors led by Adrian Harris at the ICRF's Clinical Oncology unit in Oxford will apply next week for permission to test the therapy on a small group of terminally ill patients. They will inject the switch, hooked up to IL-2, directly into melanoma secondaries that have spread in their skin. The team hopes the tumours will shrink, although no cures are expected yet.

In the US, scientists are already experimenting with other forms of gene therapy for cancer. One method is to remove some tumour cells from the patient at the time of surgery, insert a gene for an immune-stimulating protein into them, and return them to the body. Another is to remove killer T-cells from the body, insert a "warhead" gene into them and return them to the body. (Source: *New Scientist*, 6 March 1993)

"Gene gun" aims at Parkinson's

Prospects for treating Parkinson's disease with gene therapy look brighter thanks to improved methods for introducing foreign genes into brain tissue. The techniques could eliminate the ethically fraught practice of transplanting healthy brain cells from aborted foetuses into the brains of people with Parkinson's disease.

The transplanted foetal cells manufacture dopamine, a vital brain-signalling chemical that people with Parkinson's disease lose the ability to make and, as a result, lose their coordination. The new methods could make it possible to insert genes that manufacture dopamine into brain tissue taken directly from the patient, circumventing the need for foetal tissue.

Ning-Sun Yang of Agracetus, a biotechnology company in Middleton, Wisconsin, worked with collaborators from the University of Wisconsin at Madison to evaluate several techniques for inserting foreign genetic material into the brain tissue of rats. They successfully transplanted a number of genes, including one which makes tyrosine hydroxylase, a substance from which the body makes dopamine.

Yang reports that by far the most successful method tried was particle bombardment with a "gene gun". He says that it is "substantially more efficient" than conventional techniques. This gun also takes only a couple of minutes to use as opposed to the hours or days needed by alternative methods.

First, the research team coated tiny gold particles less than a micrometre across with the DNA to be inserted into the brain tissue cells. The researchers first removed brain tissue from the rat, then they propelled the particles towards the target with a shock wave generated by a powerful electrical discharge. The gold particles were blocked by a grid in front of the target, but the DNA continued and embedded itself in the brain tissue cells.

The researchers then inserted the transformed cells into rats with a version of Parkinson's disease. Disappointingly, the newly inserted genes showed very low levels of activity in their new host. Only tiny amounts of tyrosine hydroxylase, for example, were produced in rats that received the gene to make it.

However, Yang suspects that it is the trauma of the surgical procedure, not the effectiveness of the gene transfer technique, that is to blame, and he believes the technique may even kill the transplanted cells. (Source: *New Scientist*, 24 April 1993)

Arresting brain disease

Tissue from the gut transplanted into the brain could help to repair nerve and brain damage, and halt the slow degeneration seen in disorders such as Parkinson's and Alzheimer's disease. This is the hope of Geoffrey Burnstock of University College, London, who has found that nerve cells controlling the movement and digestion of food in the gut have intriguing properties. Burnstock's work focuses on the enteric nervous system, which he describes as a "small brain" in the abdomen. This is made up of 100 million neurons, or nerve cells, and operates independently of the brain. In experiments on rat tissue Burnstock found that these specialized cells encourage brain cells to regenerate, which they do not otherwise do. He has also shown that they survive and grow in the brains of rats, raising the prospect of repairing damaged brain or spinal tissue with transplanted gut cells.

He told the annual meeting of the British Association for the Advancement of Science that his system has three potential benefits. "One is that you can take the tissue from yourself," he said. "The second is that the tissue will not trigger an immunological reaction because it is your own tissue, and the other is that all the chemical messengers produced in the brain are also produced in the gut cells."

But enteric nerve cells have an added attraction. Burnstock found they also produce substances that stimulate brain cells to grow.

He hopes ultimately to treat people using his new techniques and says that extracting the necessary gut tissue would require minimal surgery. For disorders such as Parkinson's disease, in which brain function deteriorates with diminishing levels of the neurotransmitter dopamine, the aim would be to use gut cells to carry functioning dopamine genes into the brain. These genes would then

produce dopamine, making up the shortfall. The gene would be transferred into the gut cells using a harmless version of the herpes simplex virus.

If the procedure works in humans, it would sidestep the ethical objections raised by other brain implant techniques. For Parkinson's disease, these include implanting tissue taken from aborted foetuses. (Source: *New Scientist*, 11 September 1993)

Drugs take the iron out of malaria's fire

Malaria is to come under renewed attack from a class of drugs that mop up iron in the body. Trials in Africa and Thailand have already produced encouraging results and more studies are to start.

Chelating agents bind surplus iron and remove it from the blood. At present, they are used to treat diseases such as thalassaemia, in which patients accumulate surplus iron from repeated blood transfusions. Robert Hider from King's College, London explains how the agents could be developed as antimalarial drugs.

Malaria parasites make their DNA with the help of an enzyme called ribonucleotide reductase, which contains iron. Chelating agents interfere with the enzyme's activity and stop the parasites making DNA.

The most widely used chelating agent, desferrioxamine, has already been tested in malaria patients in Zambia and Thailand by a team led by Vincent Gordeuk at George Washington University in Washington DC. The drug removed the parasite from the blood and reduced symptoms in malaria sufferers. However, desferrioxamine cannot be taken by mouth, and it can have severe side effects.

Hider and his team are developing antimalarials that can be taken by mouth and target only those red blood cells infected with parasites. The parasite makes a protein which transports excess lactic acid out of the cell. Hider's team attaches a lactic acid-like molecule to the iron chelator so that the drug is attracted by the protein, specifically to infected cells.

Gordeuk and his colleagues in Zambia and the US will carry out a small trial of one of the first chelators developed by Hider, known as L1, for cerebral malaria. Cerebral malaria is the most severe form of the disease. Scientists believe excess iron builds up in the brain, prompting dangerous free radicals to kill brain cells. L1 has been widely used in the West to treat thalassaemia but it also has side effects.

Hider sees this trial as a first step, but is working on more refined chelators for trials that could start in two to three years. Gordeuk and his colleagues are also planning a larger trial of desferrioxamine in 300 children with cerebral malaria. (Source: *New Scientist*, 11 September 1993)

Therapy targets viral protein

A new treatment for AIDS under development at Purdue University in the US may some day be used to intercept the human immunodeficiency virus (HIV) before it infiltrates healthy cells. Compared with current AIDS treatments, scientists suggest that the new compound, called cosalane, may cause fewer side-effects and reduce the production of resistant viral strains.

As Mark Cushman explains, experiments with mice appear to show that cosalane inhibits the virus from killing cells at concentrations that do not kill healthy cells. It works by preventing a vital protein on the virus (gp120) from attaching to its receptor site on the cell membrane (CD4), but Cushman does not yet know whether cosalane itself binds to gp120 or CD4.

The new agent also hinders the fusion that usually takes place following attachment of the gp120 protein and it is this which is novel for such a low molecular weight, nonpeptidic compound, says Cushman. How the new agent achieves this is still unknown.

Cushman speculates that, because cosalane works in a different way to current treatments such as AZT and ddI, which inhibits the viral enzyme reverse transcriptase and suppresses proviral DNA, it may be able to suppress the virus without causing toxic side effects such as anaemia and pancreatitis. He further suggests that it might not produce resistant viral strains.

Before starting human trials, Cushman must synthesize the drug in large enough quantities and undertake toxicology studies. As a result, he estimates that it will be a year before the drug goes to clinical trials.

Cushman hit on cosalane after working with a polymer called ATA (aurintricarboxylic acid) which had potential for use as an AIDS therapy. As ATA was too complex and uncontrollable, Cushman attached a low molecular weight, chlorinated derivative to a steroid (cholestane) and came up with the promising cosalane. (Source: *Chemistry & Industry*, 6 September 1993)

Brain cancer cure

US scientists are poised to announce promising results from the first human trial to cure brain tumours using gene therapy.

The key to the gene therapy approach is the use of a so-called suicide gene from the herpes virus. Patient tumours were injected with mouse cells modified to manufacture and secrete an engineered herpes retrovirus. The treatment is selective because retroviruses only affect dividing cells and the only ones doing so in the brain are those belonging to the tumour. Once inside the tumour cells the viral genes are inserted into the host cell DNA. In the following week, patients were given the

anti-herpes drug gancyclovir to halt further tumour cell production.

French Anderson of the National Institutes of Health has said that there had been no adverse effects in the eight glial blastoma multiforme patients given therapy in December 1992. Using conventional therapies such patients tend to die within nine to 12 months of diagnosis. The patients selected by the NCI team had already been treated with other methods but these had failed. While the scientists are cautious about the potential for treating brain tumours, expectations of success are high owing to the excellent results obtained in animal studies. (Source: *Chemistry & Industry*, 6 September 1993)

HIV drug approved for clinical trial

Allelix Biopharmaceuticals Inc., Toronto, has received Canadian Government approval to proceed with Phase I human clinical trials of its drug ALX40-4C for the treatment of the human immunodeficiency virus (HIV) infection. Allelix plans to begin studies of safety and tolerance in human volunteers.

4C works by preventing HIV from using its genetic information to produce new virus particles. The specific target of the drug is a critical interaction between two virus components, the transactivator protein Tat and the RNA structure TAR. This is a different mechanism than any current HIV drug on the market or in clinical trial. It is the first drug from Allelix's research programme in gene or transcription targeted pharmaceuticals to enter clinical trials.

The company is developing antiviral agents and products to treat bone disorders. Allelix's research programmes also focus on the development of anti-inflammatories and CNS therapeutics. (Source: *News Release*, 20 September 1993)

Nuclear biotechnology

The fruits of research begun a decade ago may soon provide help in diagnosing one of the world's biggest killers - blood clots in the lungs, or pulmonary embolism. The research, a joint project by Brisbane-based Agen Biomedicals and the Australian Nuclear Science and Technology Agency (Ansto), has combined monoclonal antibodies and nuclear medicine to provide images of blood clots deep inside the body with no need for surgery.

The technique uses DD-3B6, a monoclonal antibody developed by Agen in the 1980s. DD-3B6 binds very specifically to a section of cross-linked fibrin, a protein found only in blood clots. A team at Ansto, led by Fook-Tien Lee, then developed a method to attach a radio-label, technetium-99m, directly to the antibody.

This "bioradiopharmaceutical" can be injected intravenously and is taken up by clots in the patient's body where it can be detected by measuring the gamma rays emitted.

As yet only small-scale preliminary trials have been carried out. However, these trials "repeatedly" detected clots deep in the veins of the leg, and "in a small number of cases" also located pulmonary embolisms.

The next step is to gain approval from the US Food and Drug Administration (FDA). (Source: *Chemistry & Industry*, 7 June 1993)

Photodynamic cancer therapy

New York - Surgery, radiation and chemotherapy are the three mainstays of cancer treatment. Yet photodynamic therapy (PDT) - which uses a laser and a light-activated drug to selectively kill cancer cells - may some day join this trio. Quadra Logic Technologies (QLT, Vancouver, British Columbia) recently received marketing approval in Canada to treat recurrent superficial papillary bladder cancer with its PDT drug, Photofrin.

PDT is a two-step process. QLT, for its part, initially injects patients with Photofrin. The compound-a mixture of dihematoporphyrin ethers and esters that absorb light at 630 nm - binds to low-density lipoproteins (LDL) in the blood. The greater number of LDL receptors in cancer cells causes a greater accumulation of Photofrin in these cells, compared to normal cells. QLT then illuminates the cancer cells with 630 nm light from an argon-dye-pumped laser, causing Photofrin to absorb photons and simultaneously release single atoms of oxygen that subsequently destroy cancer cells. (Extracted from *Bio/Technology*, Vol. 11, June 1993)

Texas firm will make taxol from fungus

A small Texas company has been awarded the licensing rights to help Montana State University (MSU; Bozeman, MT) researchers develop a new technique for producing taxol from a previously unknown fungus. The collaboration may lead to an improved method for making the promising cancer therapeutic, and save rare yew trees as well.

Andrea Stierle, Ph.D., an organic chemist in MSU's department of plant pathology, first discovered the yew tree that housed the unique fungus 13 years ago while hiking in Glacier National Park in north-western Montana. When the idea arose to look for a taxol-producing fungus in the bark of a yew, Dr. Stierle went back to the park and found that a fungus scraped from the bark of the yew tree she had discovered produced taxol. After three years of searching, no other yew trees from the same area of Glacier Park have been found to contain the taxol-producing fungus.

The fungus is a new species with its own genus, which the MSU scientists named *Taxomyces andreanae* (after Andrea Stierle). It was derived from the phloem of the Pacific yew (*Taxus brevifolia*).

Researchers cultured the fungus by transferring hyphal tips from water agar (on which pieces of bark had been cultured) on to modified mycological agar. The presence of taxol in three-week-old cultures was confirmed by mass spectrometry, immunochemistry, chromatography and radiochemical labelling.

The potential commercial payoff from large quantities of taxol produced by fungal cultures made the MSU discovery a hot item.

Cytoclonal plans to transform the fungus into a taxol factory and become the world's leading supplier within five years. Doing so may well require fungal genetic engineering experts. So far the fungal cultures have produced only nanogram quantities of taxol.

To be a commercial success, milligram amounts need to be churned out. One possibility for increasing production would be to isolate the part of the genome that was transferred between the tree and fungus. This section likely contains the taxol-synthesizing system that can be scaled up inside a yeast or bacterial cell.

Another approach is to look for specific regulators of the fungus. In many plant/microbe systems, a critical component of the host plant is required for the microbe to make its target compound.

While fungal manipulations are under way in Texas, Dr. Stierle will focus on the chemistry of her fungal namesake in Montana. Her goal is to find within the fungal extract a chemical cousin that will supersede taxol and eliminate toxicity problems seen in clinical trials.

Just how the genes for taxol production were transferred between this particular Montana yew and its fungus remains an ecological mystery. But finding the novel taxol-yielding fungus profoundly illustrates the need to preserve the biodiversity of ecosystems.

Fungi constitute the largest group of the earth's organisms, yet 95 per cent of them have not even been taxonomically classified. The lure of quick profits through molecular biology has forced professions like mycology to lose prestige and funding. (Extracted from *Genetic Engineering News*, 16 July 1993)

Livestock applications

Vaccine goes live on the fish farm

The world's first live vaccine for use in fish farming has just passed its initial safety trial in the west of Ireland. The vaccine protects against furunculosis, one of the most important diseases of farmed salmon and trout, and its use could considerably reduce the use of antibiotics in aquaculture. But because it is the industry's first genetically engineered product and because market prices for salmon are low, no company is so far willing to manufacture it.

Furunculosis, a virulent and usually fatal disease of salmon and trout, is caused by the bacterium *Aeromonas salmonicida*, and infection with just 10 bacteria can produce clinical symptoms. Strains of the bacteria have emerged that are resistant to more than one drug treatment.

The live vaccine, BriVax, is a mutant of *A. salmonicida*, developed jointly by Trinity College, Dublin and BioResearch Ireland, a state company established to commercialize Irish biotechnology.

The safety trial in 1992 was the first deliberate release in Ireland of a genetically modified organism. The trial found that, depending on water temperature, the vaccine persisted in the fish for between 14 and 30 days, and in the nutrient-rich sediments under the tanks for at most eight weeks.

The vaccine is currently being injected into the fish. The vaccine can be given manually using a dose gun, as is the case on many fish farms, but for large-scale applications it can be automated using a machine to sort the fish and then inject them at a rate of some 4,000 an hour. In either case, the fish are lightly anaesthetized to reduce the stress of being handled. BRI has applied for patents for the vaccine and the genetic manipulation in all the major fish farming countries and is seeking manufacturing agreements. (Source: *New Scientist*, 24 April 1993)

Agricultural applications

Fusarium control

Mogen International claims to be the first to have demonstrated fungal resistance in genetically engineered plants at commercial levels. The company claims to have achieved a high level of tolerance of *Fusarium* wilt in tests performed on tomatoes. Mogen scientists genetically engineered tomato plants of the Moneymaker variety to express genes, isolated from tobacco, that encode two types of antifungal proteins. Three to 4 weeks after inoculation with *Fusarium*, engineered lines showed only mild symptoms and subsequently recovered completely to perform as well as uninfected control plants. *Fusarium* killed all non-engineered plants and plants that contained only a gene encoding for one of the anti-fungal proteins. Mogen claims it has proved impossible to find conventional methods capable of controlling *Fusarium* in some plants. (Source: *European Chemical News*, 10 May 1993)

HPATC mass producing male-only asparagus

A research team at the Hiroshima Prefectural Agricultural Technical Centre (HPATC) has developed a biotechnological method to mass produce a high-grade male breed of asparagus plantlets for commercial cultivation.

The method broadly entails the production of an adventitious embryo through the culture of shoot growth

points. The embryos are then transferred to a medium with a high agar content for further development into plantlets. The centre plans to seek patent rights covering the culture method. (Source: *McGraw Hill's Biotechnology Newswatch*, 5 July 1993)

How blue genes could green the cotton industry

The fabric dyes used today to colour cotton are not the world's most environmentally friendly substances. Their ingredients are often hazardous and their wastes polluting, and they take time and energy to work into the cloth. But plant breeders and genetic engineers may have the means to green the industry - by producing cotton bolls that are brightly coloured to begin with.

Genetic engineers are most concerned with "blueing" the industry. Two companies are trying to develop genetically engineered cotton to make jeans blue - Agracetus, in Wisconsin, and Calgene, in northern California. Calgene is just starting its blue cotton project; Agracetus's effort is further along - and is only one of many plans the company has for cotton. It also wants to engineer stronger, longer, finer, warmer and wrinkle-free fibres.

The genetic engineers plan to insert into cotton plants the genes that are responsible for the production of the blue colour in the indigo plant - the source of blue dye until a cheaper synthetic method of making it was discovered. The genes would be engineered in such a way that they would be active only in the cotton fibres, so only the cotton boll would turn blue.

Genetic engineering is not the only path to coloured cotton: naturally coloured varieties already exist. Sally Fox stumbled across these in the 1980s. She was working with a cotton breeder in California, trying to breed insect-resistant varieties, and one of their pest-resistant stocks also happened to have rust-coloured fibres. "The fibre of these cotton seeds was very difficult to spin - quite short, and rather coarse," says Fox. "But the colour was beautiful."

Fox knew that naturally coloured cotton was hand-spun and woven around the world, but that no one had ever bred a strain that could be spun by commercial machines. In her spare time, she began crossing the rust-coloured cotton with a high-quality, long-fibred, white variety, hoping to create cotton bolls with the colour of one and the fibre of the other.

She moved to California's San Joaquin valley - one of the country's prime cotton-growing regions - to expand her breeding efforts, and by 1988 she had produced the first machine-spinnable coloured cotton.

Today, Fox has several machine-spinnable browns and greens, and is working on improving fibre quality in a range of others: greys, oranges, yellows, even a mauve. Blue, however, has eluded her and so far remains the province of the genetic engineers. Cotton from Fox's company, Natural Cotton Colours, is being woven into shirts, jackets, sheets and socks for major clothing manufacturers, including - despite the absence of blue - Levi Strauss. (Source: *New Scientist*, 31 July 1993)

Slug control

Nematodes could provide farmers and gardeners with the ultimate weapon against slugs. One species of nematode, less than a millimetre long, is a natural soil dweller and preys exclusively on molluscs, such as slugs and snails.

The Agricultural Genetics Company (AGC) in Cambridge plans to package the live worms in clay powder. Farmers and gardeners will be able to apply the worms as a spray after mixing the powder with water. "The mixture will last three to six months if stored in a fridge," says Paul Rodgers, the company's research manager.

It is the first known example of a nematode that will kill slugs," he says. The worms burrow into slugs through a tiny pore on the back of the mollusc. They carry bacteria on their bodies which kill the slug, and the worms reproduce in the cadaver, feeding on the rich nutrients in the dead mollusc. The company has worked out a way of mass-producing the worms in fermenters. The worms may be on sale within two to three years.

Rodgers hopes that the new formulation will be popular because it only kills slugs. He points out that slug pellets, containing chemicals such as metaldehyde and methiocarb, are the only existing means to control slugs, but each year the pellets kill a number of domestic cats and dogs.

The company is developing the worm-based formulation in collaboration with the Agricultural and Food Research Council's Institute of Arable Crops Research in Long Ashton near Bristol. Rodgers says the worms will not become pests themselves, and will largely die out in winter. (Source: *New Scientist*, 6 February 1993)

Pesticide resistance of coca plant

Man has long known the stimulating and potentially medicinal properties of cocaine, but its botanical *raison d'etre* has remained a mystery. Now, American scientists are suggesting that cocaine - found in high concentrations in the leaves of coca plants - provides the plant with a natural defence against insects. They also claim that new information on how cocaine works in insects will provide a basis for new, safer pesticides.

Plant biologists have long observed that coca plants remain relatively pest-free despite the fact that they often have tender new leaves which emerge after the plant is stripped by growers. James Nathanson and his colleagues at the Massachusetts General Hospital in Boston believe

this is because the amount of cocaine normally found in coca leaves is enough to be insecticidal.

Nathanson sprayed small amounts of cocaine on tomato leaves and found that insects placed on these leaves stopped feeding, became hyperactive and, at higher concentrations of cocaine, died within 24 hours. Cocaine also killed mosquito larvae, he reports.

Nathanson believes that cocaine blocks the removal of an important neurotransmitter, octopamine, which passes messages between nerve cells in insects. Blocking the removal of octopamine (which normally occurs by transport of this transmitter from the synapse into cells) results in an excess of octopamine. "The effect is much like an overdose of adrenalin in humans - anorexia, hyperactivity and even death," says Nathanson.

"It is possible that a chemical very specific for blocking octopamine in insects could be a very safe type of pesticide," speculates Nathanson, "as it would have very little effect in humans." However, Nathanson stresses that cocaine itself would make a very poor pesticide because it blocks removal of both insect and human neurotransmitters. (Source: *Chemistry & Industry*, 1 November 1993)

Japanese modify more edible rice

Rice that is more edible and resistant to important pests and diseases is on its way. Researchers at the Mitsubishi Kasei-funded Plantech Research Institute have used antisense technology to produce rice that contains less amylose; introduced genes to make rice resistant to two major rice pests; and used viral coat protein genes to make rice resist the rice stripe virus.

The amylose content of rice starch is a major determinant of the rice's eating quality. Amylose is regulated by the "waxy" gene which encodes for a starch grain-based glycosyl transferase. Plantech's Ko Shimamoto revealed that antisense constructs block expression of the waxy gene and so lower the amylose content of the rice. More importantly, the antisense-transformed rice plants are as viable as wild types and much more viable than existing waxy mutants.

Using the classical *Bacillus thuringiensis (Bt)* endotoxin gene and protoplast generation technology, Shimamoto and colleagues have engineered rice to resist both the brown plant hopper and the striped stemborer. The Plantech researchers modified the *Bt* codon to ensure high expression of the toxin gene in rice and report that the resistance trait is stably inherited. Although the rice is now ready to be tested in field trials, there are no plans to do so at the moment.

Full scale trials of virus-resistant rice engineered by the Japanese team are, however, likely to take place next year. To engineer rice to resist the rice stripe virus, the team has introduced genes coding for the virus coat protein into rice. "Transgenic plants efficiently expressed the coat protein and exhibited resistance against virus infection," Shimamoto noted. Small-scale trials have already been conducted this year with the transgenics and he confirmed that the testing process will be stepped up some time next year.

Plants that have been genetically modified to resist pests and disease have so far been given genes from bacterial and viral sources. Now, attention is focusing on plants which have a natural ability to resist disease and pests, because genes conferring these traits should be easier to express in transformed plants.

Richard Michelmore and colleagues at the University of California, Davis, are investigating the genetic basis of disease resistance in lettuce, focusing on downy mildew caused by the fungus *Bremia lactucae*. Parallel genetic studies on host and pathogen have shown that at least 13 dominant genes for resistance in lettuce are matched by avirulence genes in *B. lactucae*. (Source: *Chemistry & Industry*, 6 September 1993)

Improving biocontrol

Biocontrol - using micro-organisms to control plant disease - may receive a boost thanks to the work Alan Paau, Mari Bennett and Lori Graham at W R Grace in New York. The technique is often overlooked, they say, because it is "perceived by some as not effective, reliable and cost-effective enough for present large-scale agricultural use." Now, however, the Grace team have found a way to increase greatly the activity of fungal biocontrol agents.

The researchers have been looking at controlling the two common fungal diseases: root rot, a disease of stone fruits and cereals which is caused by the *Rhizoctonia* fungus, and "damping off", caused by the *Pythium* fungus, which affects almost all crop plants, causing seeds and stems to collapse. While working with two other fungi *Gliocladium virens* and *Trichoderma*, to try to suppress these pathogens, they found quite unexpectedly that exposing these other fungi to "deactivated or dead pathogens" enhances their activity. The fungi can be exposed to the pathogens, which are deactivated by heat treatment, either during cultivation or during storage.

The enhancement claimed by the researchers is quite dramatic; in some cases inactive fungi can even become active. They quote tests using soil exposed to active *Pythium* which show that, compared to an untreated control sample, treatment with unenhanced *Trichoderma* caused 2.6 per cent more crops to be infected. However, spraying the soil with the enhanced agent led to 12.6 per cent fewer crops being infected, they claim.

Despite their apparent success, the researchers are quite unsure what is actually going on. They do not yet understand how *Gliocladium* or *Trichoderma* suppress the pathogens, they say, nor do they claim to know why their

enhancement technique works. They believe that somehow "exposure to the fungal pathogen excites or induces the biocontrol to more effectively utilize whatever the mechanism is that hinders pathogen growth" (Source: *Chemistry & Industry*, 21 June 1993)

Synthetic forest tree seeds

Dr. Steve Attree, a professional research associate in Professor Larry Fowke's laboratory in the Biology Department, University of Saskatchewan, is developing new methods for propagating Canadian forest trees (conifers) using somatic embryogenesis. This involves removing single embryos from spruce seeds and culturing them on a specifically developed nutrient medium. In culture these seed embryos produce a rapidly dividing mass of tissue composed of large numbers of immature somatic embryos (non-sexual embryos) each one genetically identical to the original seed embryo. Thus from a single seed embryo with particular characteristics, potentially unlimited numbers of identical somatic embryos can be cloned.

Treatment of the immature embryos with a specific plant growth regulator causes them to mature and they can then be germinated and transferred to soil to form young trees. Until recently this process of maturation was inefficient and yielded rather poor quality embryos. Over the last three years Dr. Attree has dramatically improved the maturation of conifer somatic embryos. He has been able to synchronize maturation and produce large numbers of very high quality embryos. Biochemical and structural studies indicate that the mature somatic embryos have as much storage reserves (fats and proteins) as a normal seed embryo. A new method involving bioreactors has been developed to scale-up this maturation process so that thousands of embryos can be matured in a single chamber.

Dr. Attree further improved the culture system when he demonstrated that the high quality mature embryos could survive drying and germinated at high frequencies. Drying of somatic embryos mimics the normal developmental process occurring in seeds. Dry somatic embryos can be stored for prolonged periods particularly when frozen. Thus embryos produced throughout the year can be stored and germinated in the spring to provide cloned plants of uniform size. Dr. Attree found that the dry embryos can also be encapsulated with a protective layer to yield dry "synthetic seeds" capable of being handled by mechanical seeders. In future, seed coats for such synthetic seeds will likely contain nutrients, growth regulators and fungicides to promote early seedling establishment in the soil.

The new methods for maturation, desiccation and encapsulation of somatic embryos of white spruce form the basis of a patent filed December 1991 in the USA and subsequently world-wide by University of Saskatchewan Technologies Inc. These methods should be valuable to the forest industry for propagating specific trees with known characteristics such as improved wood quality, disease resistance and environmental tolerance. They will also facilitate handling and storage of synthetic conifer seeds.

Further information available from Professor Larry Fowke, Department of Biology, University of Saskatchewan. (Source: *The AgBiotech Bulletin*, Vol 1, No. 3, May/June 1993)

Integrated Pest Management - A safer plant protection option

Evidence continues to accumulate which offers indications that several of the commonly used pesticides alter the biological ecosystems, initiate a chain of non-target reactions, and exert significant influence on micro-organisms other than those they are supposed to suppress. Assessment of biocidal effects is naturally necessary to avoid environmental disruptions such as enhancement of non-target diseases and pests, and suppression of microbial antagonists which may act to hold non-target pests and disease organisms under check.

We live in a world in which we have become heavily dependent on chemicals. The use of pesticide chemicals has virtually become an essential adjunct to modern agriculture. But then with any new such technology, there is over-use and misuse, and despite the appearance of pest resistance and recognition of some adverse effects of non-target organisms, little serious thought has been given to the potential long-term consequences of pesticidal use. In fact, Rachel Carson was probably one of the first to emphatically express the view that planet Earth was a finite entity, and that Man and biosphere would be doomed, unless somehow we learned quickly to control the technological ability. Her classic book "Silent Spring" in 1962 almost overnight shifted the balance.

In recent years several pesticidal side effects have been noted particularly in terms of destruction of induced antagonists, development of pathogen resistance to pesticidal applications, disease insurgence, and above all general ecological disruptions. This awareness has led to the concept of "Integrated Pest Management" (IPM). Implicit in its concept is development and utilization of all sustainable techniques and methods in as compatible a manner as possible for an overall and efficient management of the pests and pathogens. Conceptually, it involves the selection, integration and implementation of control based on predicted economic, ecological and sociological constraints. Researches on minimal use of systemic pesticides and maximum reliance on natural and regulatory mechanisms to maintain pests and diseases, below the level at which they cause economic damage is being currently strengthened. This approach has definite advantages over chemical options, particularly where there is a possibility of "managing" rather than "controlling" the potential pests and diseases to achieve a balance, and has a self-regulating

system rather than one maintained by a series of crisis-driven unsustainable control measures.

Realizing the magnitude of the side-effects exerted by a range of pesticides, the Indian Council of Agricultural Research (ICAR) has recently established a National Centre for Integrated Pest Management. This national centre envisages utilizing various physical, chemical and biological means of control and modification techniques in a compatible manner. The integration of control tactics such as plant host resistance, biological control agents, chemicals and culture practices into a "best mix" for keeping the devastating pests and pathogens under check to an acceptable level, is the basic IPM doctrine being followed. Such a system shall naturally include different levels of biological interactions, ranging from molecular biology to spatial analysis of population across geographical regions. The centre has initiated the establishment of an IPM data bank to serve as a national facility, and also to facilitate development of sound prediction models for major pests and diseases affecting crops of economic importance.

Further information available from Dr. Bushan L. Jalali, Director, National Centre for Integrated Pest Management (ICAR), 646 Sector 21-A, Badkhal Road, Faridabad 121 001. (Source: *Biobytes*, Vol. 2, No. 1, 1993)

Food and food processing

Progress made in food users of biosensors

Products of biotechnology, ranging from monoclonal antibodies to enzymes, are successfully being harnessed to develop selective, sensitive sensors for analysis of food contaminants. But despite this progress, food safety applications of biotechnology have been limited by, on one hand, resistance of food processors to new techniques and, on the other, opposition of consumers to biotechnology in general. The application of biotechnology to food analysis has progressed in recent years, but acceptance of the new techniques has lagged behind their development.

Biotechnology-based diagnostics exist or are being developed for analysing contaminants at every stage of the process of bringing food to market. Although traditional analytical chemistry and microbiological techniques have met the needs for food analysis, the methods are limited by complexity, time required for analysis, and cost.

However, scientists are using biotechnology to address these limitations. Immunoassays have been developed for a variety of food components and contaminants including amino acids, sugars, alcohols, pesticides, and organic compounds. Nucleic acid probes focus on identifying pathogenic micro-organisms in food. Biosensors are being developed to detect food components and contaminants, as well as to determine quality variables such as odour and taste.

Until recently, new methods for monitoring food safety have been primarily novel applications of chromatography well known to the analytical chemist. These conventional approaches are important and represent the officially recognized methods of analysis in almost all instances.

By contrast, immunoassay techniques hold promise but have yet to prove themselves to food chemists. Establishing the utility of such techniques will require tested applications; data reflecting precision, accuracy, and ruggedness; and above all, correlation with existing HPLC or gas chromatographic methods.

The conventional technology for detecting Salmonella in food requires five days to ensure that a food sample is free from bacteria. By coupling "immunocapture" technology - which uses antibodies to capture whole bacterial cells - with standard enzyme-linked immunosorbent assay (ELISA) technology, this time can be reduced to 24 to 48 hours. (Abstracted with permission from *Chemical & Engineering News*, 19 April 1993, p. 37. Copyright (1993) American Chemical Society)

Improved potato chips

Advanced Technologies, based in the Cambridge Science Park, is using biotechnology to reduce sucrose in potatoes. Potato crisps and chips get too brown when they are fried if the original potato contains too much sugar. This happens when sucrose accumulates in potatoes which are stored at low temperature.

As AT's European patent application (530 978) explains, sucrose migrates to the tubers after being formed in the leaves by an enzyme called sucrose phosphate synthase, or SPS. The company isolated the SPS protein, and analysed its amino acid sequence to obtain a DNA clone of the gene that instructs the plant to make SPS. The modified version of the cloned gene was then introduced into the plant by *Agrobacterium tumefaciens* bacteria. The modified SPS decreases sucrose synthesis - so there is less sucrose to go to the tubers, and less browning on frying. (Source: *New Scientist*, 28 August 1993)

New whey for yoghurt

Cornell University scientists think they can get rid of the clear liquid on yoghurt or fromage frais by manipulating the milk protein normally found in whey. This would solve an annoying food processing problem and increase milk's economic value.

Carl Batt and his colleagues are among the first to use a "rational design approach" to alter the properties of a milk protein and create "minigenes" for transgenic cattle. Using a crystal structure of the whey protein beta-lactoglobulin - the only existing crystal structure of a milk protein - the team selected the precise targets they wanted to modify. They substituted one naturally occurring amino

acid for another, using a technique called site-directed mutagenesis.

To increase the yoghurt's tendency to form a gel, the team focused on the thiol group in the protein, explains Richard Brandon, a food scientist working with Batt. After heating, beta-lactoglobulin forms a gel when it cools down; and the "freer" the thiol group, the greater the gelling tendency of the protein.

"We have every reason to believe that these genes eventually can be expressed in cows, which would open new markets for the use of whey proteins." Beta-lactoglobulin is found in the milk of many ruminants. It is thought to deliver the vitamin retinol, held in an interior cavity of the protein, to nursing offspring.

The modified protein reduces the separation of whey, which accounts for the pools on top of yoghurt, by as much as 83 per cent, claims Batt. This work should cut the costs of making yoghurt, he adds. Yoghurt made with small amounts of modified protein forms a gel six to ten times easier than ordinary yoghurt, so eliminating the need for adding starch. Plus the processing temperature can be lowered from 85° C to 70° C or less, and curd takes less time to form.

Batt also thinks that the modified proteins may be developed as transport agents for pharmaceuticals. Site-directed modifications to the interior cavity may permit the protein to carry blood-clotting factors or insulin through the stomach, he says. (Source: *Chemistry & Industry*, 21 March 1994)

Chemical applications

Starch-based biodegradable plastic

Japan Corn Starch Co. Ltd. and an American business development firm, Grand River Technologies (GRT) based in Lansing, Michigan, have started developing and producing starch-based biodegradable plastic in a joint venture. Unlike traditional plastics made from petroleum-based oil, the new plastic readily decomposes and can be used as a compost fertilizer for corn. No scarcity of resources is likely since the plastic is made from corn.

The new plastic is formed through a special chemical and physical treatment developed by Japan Corn Starch Co. Ltd. in cooperation with Michigan Biotechnology Institute chemist Ramani Narayan. It has remarkably high resistance to water compared to corn-based plastic made through the conventional method, but decomposes into water and carbon dioxide completely in 28 days when buried in earth or a compost bin. The plastic has the same uses as petroleum-based plastic but is pollution-free since it contains no petroleum components.

The companies have established a joint-venture enterprise based in Lansing, Michigan, to mass-produce low-priced products based on the new plastic. For the next three years, the enterprise will try to improve treatment technology and the production process to reduce the production cost from the present ¥500-1,000 to ¥200-250 per kilogram, which is comparable with plastic made from petroleum-based oil, to mass-produce disposable dishes or cups, film, paper coating, drinking bottles, etc.

A patent for the new plastic is already pending in major countries around the world.

Further information available from: Japan Corn Starch Company Ltd., 22F Nagoya Tokio Kaijo Bldg. 20-19, Marunouchi 2-chome, Naka-ku, Nagoya 460; Tel.: +81-52-211-2011; Fax: +81-52-231-2024. (Source: *JETRO*, December 1993)

Optical resolution of 1,2-Propanediol with microbes

Daicel Chemical Industries Co. Ltd. has established a new technology for manufacturing optically active 1,2-propanediol using microbes. The microbes are used for recovering the target optically active 1,2-propanediol (by screening) from inexpensive racemic 1,2-propanediol obtained through ordinary chemical synthesis.

1,2-propanediol is a biologically active substance used as the raw material for the synthesis of antibacterial agents, but for use as a medical raw material, it will be necessary to separate the optically active S-type and R-type isomers. The 1,2-propanediol obtained through ordinary chemical synthesis is a racemic type consisting of a mixture of S-type and R-type isomers, and the present method of separation of either of the optical isomers by chemical synthesis is quite expensive.

Applying the new technology, it will be possible to recover isomers with microbes which consume only one type of isomer from an inexpensive racemic substance. Using these microbes enables S-type and R-type isomers to be recovered at a high ratio of 97 per cent at an optical purity of 98 per cent and up. The new technology is still under fundamental research, but may allow optically active 1,2-propanediol to be manufactured much cheaper than before.

This microbe, which devours a single type of isomer efficiently, belongs to the genus *Pseudomonas* and was discovered by screening soil at 883 points in Tsukuba City and other parts of Ibaraki Prefecture.

Further information available from: Daicel Chemical Industries Co. Ltd., Tsukuba Research Institute, 27 Muyukigaoka, Tsukuba City, Ibaraki Pref. 305; Tel.: +81-298-56-1322; Fax: +81-298-56-1309. (Source: *JETRO*, December 1993)

Industrial microbiology

Chitin removes textile dyes from wastewater

Chitin can be used to remove colour from wastewater discharges at textile dyeing operations, according to researchers at North Carolina State University College of Textiles, Raleigh. The research team, headed by Samuel Hudson, as associate professor of textile engineering, chemistry, and science, and Brent Smith, a professor of textile chemistry, uses a bulk powdered form of the plentiful natural polymer, derived from crab shells, as a filtering agent in a laboratory decolourization module. "We're finding out that a small amount of chitin powder will decolourize a lot of waste product, absorbing up to 20 per cent of its weight in dye," Hudson says. "At $120 a ton, the use of bulk chitin powder is a cheap way to retrieve the colour from wastewater." The dye-laden chitin powder, he adds, could be recycled for use in packaging materials or for commercial fuel logs. In North Carolina, 5 million pounds of fabric are dyed daily, and textile-related industries discharge more than 64 million gallons of water directly into the environment each day. The researchers note that regulation of dyes in wastewater is not yet mandated, but some mills are required to monitor and report colour in their wastewater discharge. (Reprinted with permission from *Chemical & Engineering News*, 12 July 1993, p. 24. Copyright (1993) American Chemical Society)

Bioreactor makes bone marrow and blood cells

A team of scientists led by David Wu of the University of Rochester has built a bioreactor that makes bone marrow and blood cells on demand.

The bioreactor consists of a spongy material (usually a polycarbonate plastic) and a small amount of bovine collagen to encourage initial cell growth. As the reactor is perfused with nutrient medium, bone marrow cells begin to grow in clusters around the holes in the spongy material.

The cells grow in clumps similar to those in the body, unlike cells grown in flasks, which lie in just two dimensions, explains Wu. Harvesting is simple: most of the cells can simply be shaken from the sponge.

This is not the first bioreactor to produce blood cells - another, developed by a Californian company called Advanced Tissue Growth, uses nylon mesh instead of a sponge. However, Wu believes his reactor is a more accurate reflection of nature.

Wu says his group's artificial bone marrow contains most of the stages and types of cells found in natural marrow. And he believes the reactor appears to preserve the stem cells, "the mother of all blood cells."

Medical applications for the bioreactor's products could include transplants of bone marrow for leukaemia patients. At present, about a quart of bone marrow must be painfully harvested from a donor for each transplant. Other uses could be blood transfusions, and treating blood disorders like haemophilia. (Source: *Chemistry & Industry*, 19 April 1993)

Biodegradable plastic using lactic acid

The Shimadzu Corporation developed production technology that uses lactic acid as a raw material. Shimadzu will begin to operate a pilot plant that will produce 100 tons annually to provide samples. In addition, Shimadzu will cooperate and form affiliations with associated businesses to develop practical applications for the plastic. Shimadzu plans to construct a mass production plant with an annual output of 100,000 tons by 1995 and set up an organization for mass production.

Shimadzu began research on lactic acid fermentation in 1985 and in 1992 marketed a mould-removing agent and a plant activating agent.

The biodegradable plastic developed by Shimadzu Corporation uses lactic acid produced by lactobacillus as raw material. It is completely broken down to water and carbon dioxide by microbes in the soil, and even when it is incinerated, little energy is required for combustion, and it does not produce nitrogen oxides. It therefore has excellent properties from the standpoint of environmental protection. In terms of strength and transparency, it eclipses other biodegradable plastics, and when compared with plastics made from petroleum, its performance is just as good or better.

In addition, because it can be mass-produced in a continuous process from lactic acid fermentation to pellets, the plastic is expected to sell for about ¥800 per kilogram after mass production begins (present commercial products are priced from ¥800 to ¥2,000).

Possible commercial products include film, filaments, injection-moulded products, etc. More specifically, in terms of strength it is about four times stronger than polyethylene at 20 kg/mm^2, it has excellent transparency, and looks promising as a packaging material. Other main features include: (1) Its regeneration cycle (synthesis → decomposition → synthesis) occurs as a cycle in nature, and it has excellent properties in terms of preserving the environment. (2) It degrades in soil or water in one year. It is possible to adjust the time needed for decomposition from two months to two years through the use of additives. (3) Oligo-lactic acids, which are the intermediate products of degradation, actively promote plant growth. (4) It has a high melting point of 185° C. (5) It is easy to mould (just as easy as plastic from petroleum) and can be used for a variety of products. (Extracted from *Shokuhin Kogyo*, 15 March 1993)

Energy and environmental applications

Biofuel developed from sewage algae

Many different methods have been considered in the production of biofuels: ethanol from sugar beet and wheat, methanol from wood, and vegetable oil from colza, but who would have thought of fueling a diesel engine with algae growing in sewage water? D.P. Jenkins of West of England University in Bristol, UK is trying to achieve just this. A 25-kW prototype is already in operation. The chosen algae is chlorella, a green monocellular algae whose photosynthesis is extremely rapid. After having been dried and ground, it directly fuels the engine, while the carbon dioxide produced in the combustion is recycled in order to improve the rate of photosynthesis.

According to Jenkins and his team, this process should allow for the competitive production of electricity. In order to produce the algae economically and in sufficient quantities, the researchers are planning to link a bioreactor, the Biocoil, developed by the London-based company Biotechna, to the generator. This type of bioreactor is composed of a transparent tube approximately five metres in length within which the algae circulate in a nutritive liquid such as waste water. The algae then develop through photosynthesis. One Biocoil unit should be able to produce up to 15 (metric) tons of algae per year. A part of the algae produced would then be dried and ground to obtain a particle size smaller than 50 microns in diameter before being used as fuel. The size of the particles, like the cetane index in other fuels, constitutes a characteristic element of the new fuel. By varying the diameter of the particles (from 40 to 70 microns), the efficiency of the engine is directly affected. The engine is started up with diesel and then gradually switches to chlorella. Once the engine is running smoothly, the algae make up 95 per cent of the fuel. In the event of problems it is possible to revert to a supply of pure diesel. One production plant consisting of several Biocoil modules could have a capacity of 200 kW to 2 MW. The research was financed by the UK Department of Trade and Industry and Bristol University. A company is in the process of being set up in order to market Jenkins' process and negotiations are under way with a number of different industrial partners. (Source: *La Recherche*, June 1993)

DuPont bioremediates chlorinated solvents

DuPont says it has developed a simple process to bioremediate chlorinated hydrocarbons. The method, says senior consultant David Ellis, is the first biological way to bring solvent-contaminated groundwater up to drinking water standards. DuPont tested the technique on a selected area at its Victoria, TX site and plans to complete cleaning of the 10-acre contamination there. When naturally occurring micro-organisms in the groundwater are fed sodium benzoate and sulphate, they proliferate and use up available oxygen, says Ellis. The anaerobic condition prompts them to use the contaminants as oxygen substitutes. The bacteria consume one chlorine atom at a time; perchloroethylene gets chewed down to trichloroethylene, then dicloroethylene, vinyl chloride, and finally ethylene. Ellis says the technique has worked on 85 per cent of tested sites. Performance depends on the presence of the required micro-organism. (Source: *Chemical Week*, 21 April 1993)

Microbial waste treatment expected to boom over next five years

Of the estimated 145 million tons of municipal waste generated in the US each year, nearly 65 per cent (paper, yard and food wastes) is considered easily biodegradable and therefore especially amenable to biological treatment. Another 16 per cent (rubber, leather and plastic waste) is considered to be "potentially biodegradable". Currently, only a small fraction of the potential market is treated by utilizing biotechnology. Sewage treatment, for instance, is based upon the enhancement of naturally-occurring human bacteria. Moreover, without biological contractors, trickling filters or various kinds of digesters, it probably would be prohibitively expensive to purify municipal and commercial wastewater. According to a Business Communications Co. (BCC) study, *Bioremediation of Hazardous Wastes, Wastewater, and Municipal Waste*, micro-organisms are responsible for much of the natural degradation that slowly remediates contaminants released into the environment.

The specific mechanisms involved include biodegradation (often through the biosynthesis of enzymes), accumulation, biologically-induced precipitation, bio-enhanced filtration, formation of biological barriers and the bioregeneration of granular activated carbon. Any of these methods have the potential to be improved via genetically altering the micro-organisms involved. Currently, micro-organisms are being "improved" through selective breeding.

By 1998, the market is predicted to rise to over $1.4 billion representing a 4.9 per cent average annual growth rate.

The key to biotechnology's use in environmental management is the micro-organisms used. In many cases, naturally occurring microbes are used. However, proprietary microbial cultures are a well-established and growing market worth $39 million in 1992. Over the next five years, the market for waste treatment microbial cultures should grow at nearly 17 per cent to reach $100 million by 1998.

Landfarming - especially oilfield waste landfarming - represents a large market for environmental remediation via biotechnology. The oilfield has long relied on "landfarming" the hydrocarbon-rich contaminants produced during petroleum recovery. During landfarming, the wastes are buried and exposed to the natural oil-degrading microbes present in soil

Recently, it has become possible to biodegrade or otherwise treat a wider variety of hazardous wastes,

including oils, fuels, solvents, pesticides and even heavy metals. Advanced microbial treatment includes a variety of techniques involving bacteria, fungi, or other micro-organisms. Microbial action can also convert "wastes" into energy through biodegradation and fermentation. It is now possible to "mine" old municipal waste dumps for methane and in future it is possible that municipal waste will be converted directly into fuel without air-polluting incineration. Composting is another "low tech" microbial waste treatment. Both sludges and biodegradable solid wastes can be composted. Composting yard wastes is an especially attractive option since it reduces air pollution and does not use municipal landfill space. The solid waste composting operating cost market is expected to grow to $150 million by 1998. The growth will significantly increase the size and sophistication of the solid waste composting infrastructure. (Source: *Biotechnology Bulletin*, May 1993)

Energy and environmental applications

Success in Alaskan clean-up

Cleaning up oil spills by encouraging oil-degrading bacteria and fungi to grow was first suggested 21 years ago. Now, with the publication of encouraging results of the bioremediation technique's first large-scale test - the *Exxon Valdez* disaster - it appears to have truly come of age.

The *Exxon Valdez* ran aground at Prince William Sound, Alaska, in March 1989, spilling 33,000 tons of crude oil across 2,000 kilometres of rocky, hostile shore-line. A team from Exxon, the University of Louisville and West Virginia University used bioremediation, then untried on a large-scale incident, to clean up the mess.

The team treated the beaches with two fertilizers: one an oil-soluble liquid (*Inipol EAP 22*), the other in slow-release granules (*Customblen*). In all, 50 tons of nitrogen and 5 tons of phosphorus were spread across the beaches in the summers of 1989-92.

These fertilizers encouraged bacterial growth on the beaches to increase 18-fold, says the team; about a tenth of the bacteria were hydrocarbon degraders. The effects of this were startling: the bacteria broke down 44-90 per cent of the different hydrocarbons in the oil, with results visible from the air after only a fortnight.

The team monitored the toxicity of the seawater and the effects on plankton, and claims that "no adverse effects were found". However, the technique will not work on highly polar organic compounds, on oil that has already been degraded, or in areas with a low oxygen supply, such as mud flats or marshes.

Another drawback is that certain compounds in crude oil and diesel absorb on to organic matter in soil and sediment and would resist degradation. Meanwhile, the bioremediation model built up by the team is likely to be extremely valuable in the planning of future clean-up operations. (Source: *Chemistry & Industry*, 4 April 1994)

Biofilters spruce up sewage for the Seine

Parisians are worred about the water quality of the Seine. In May 1992 hundreds of tons of fish died after a storm flushed untreated sewage into the river. Bacteria in the sewage consumed so much oxygen that the fish suffocated. Now two prototype oxygen pumping systems are breathing life into the river to prevent it happening again.

A better solution would be to prevent sewage overflowing and escaping into the river. The government of the Paris region has embarked on a project worth 9 billion francs (£1.1 billion) to tackle the problem. As part of the project, the government has commissioned a new type of sewage treatment plant for the suburb of Colombes that uses bacteria in biofilters to treat sewage, and should never overflow.

There is a shortage of land along the river bank at Colombes, and the biofilter system, which costs 40 per cent more than a traditional plant, was chosen because it takes up only about 60 per cent of the space. The Colombes plant could serve as a model for other large cities where construction space is limited. The plant is being built by two French companies, OTV and Degremont. It is not expected to be finished for at least five years, and will cost 2 billion francs.

Biofilters were invented about 10 years ago. They have been used before on small treatment plants, but never on the scale planned for Colombes. They are derived from "trickling filters", beds of volcanic rock on which bacteria grow and break down pollutants as water trickles through.

In conventional treatment works, oxygen is pumped through sewage in large basins where bacteria eat the pollutants. After about 16 hours the water and resulting sludge, which includes some bacteria, are fed into a decanting basin. Over about four hours the solids sink to the bottom, leaving the water cleaner, and are recycled back into the first tank.

Water entering the Colombes plant will flow through a series of three biofilters at a rate of 2-8 cubic metres per second. The filters are packed with clay beads that carry bacteria fixed onto their surface. The first filter carries bacteria that feed on organic pollutants. Bacteria on the second filter transform ammonia in the water into oxygen-containing nitrates. In the third filter, bacteria living in an oxygen-free environment remove oxygen from these compounds, releasing nitrogen gas. The whole process takes three hours.

The Colombes plant will have the capacity to treat four times as much water as it normally receives in dry weather. During very heavy rainstorms, the three biofilters will work in parallel, each receiving one third of the flow.

Under these conditions, bacteria feeding on carbon-containing pollutants and ammonia will take over in all three filters. Nitrates will not then be treated, but they are less harmful than ammonia, which depletes oxygen and kills fish if it escapes into the river.

The Colombes plant will remove phosphates by adding chemicals to precipitate them out. The system is one fifth the size of existing methods, and produces phosphate sludge that is three times as concentrated. If demand stays at current levels, all the 80 tons of dry sludge produced by the plant each day will be used on nearby farmland. (Source: *New Scientist*, 10 April 1993)

Gene cloning picks up something nasty on the beach

The conventional method for testing water quality is to sample the sea water and wait for bacteria found in sewage to grow on Petri dishes. This process is slow and it can takes days to identify if there is a risk and longer to give the all-clear once it is safe to surf again.

Clifford Brunk, a molecular biologist from the University of California at Los Angeles, and his colleague David Chapman, a marine biologist, are developing a faster, better way to monitor bacterial and viral pollution in sea water in just a few hours, using the latest tools in molecular biology. Southern California's often filthy water is not unique, says Chapman. "This technique is applicable to any potentially polluted water."

The new methods exploit a technique called the polymerase chain reaction (PCR). Traditional tests for water quality monitor just a few bacteria, notably *Escherichia coli*, which is abundant in sewage. If *E. coli* is there, technicians assume that nastier bacteria and viruses could be lurking too. But some pathogens seem to live longer than *E. coli*, and many living *E. coli* stubbornly refuse to grow in culture, so information from culture tests is limited.

The first step of the new method is to filter the bacteria from the sea water. Then the technicians use enzymes, detergents and freeze-thawing to burst the bacteria open and extract their DNA.

Next, they use PCR to produce millions of copies of a precise segment of bacterial DNA, which is common to many bacteria but which has variations unique to particular strains. This is done by adding tiny genetic markers which bind to each end of the key segment. A DNA-synthesizing enzyme is then used to copy the marked segment over and over again.

The segment of DNA can also be used to identify individual strains of bacteria in the sample. The exact pattern of bases in the segment differs in each strain of bacteria. Technicians then use enzymes to cut the segment into fragments characteristic of different strains of bacteria.

The fragments are identified by a technique called electrophoresis. The fragments are drawn through a gel by an electric field and different sized chunks travel different distances. The result - which is analysed by computer - indicates which bacteria are present in the water, and how much there is of each one.

The procedure works well in the laboratory for about a dozen types of bacteria, say Chapman and Brunk, and they are now transferring the method to the field. Meanwhile, the nearby Orange County Sanitation Districts are also using PCR to detect dangerous viruses in sewage, like the one that causes hepatitis A. (Source: *New Scientist*, 24 April 1993)

Weeding out the troublemakers

Gardeners waging war against weeds in their flower-beds may be aghast to learn that US scientists are cultivating them - to clean up contaminants from industrial sites.

Scott Cunningham from DuPont has identified two weeds, hemp dogbane and common ragweed, that may be used on lead-contaminated soil. This process, where green plants remove, collect or render pollutants harmless, is called phytoremediation.

Cunningham found a common ragweed growing on a lead-contaminated site which had lead concentrations of 700ppm in its leaves and 2,000ppm in its stem. "Over the years, the common ragweed has learnt to pick up and tolerate lead and is now different to other ragweeds," he says. Similarly, he found hemp dogbane on the same site with lead concentrations of 400ppm in its sap.

Reclaiming the lead is simple, and energy-efficient, claims Cunningham: just collect the weeds, throw them in a kiln and shut the door. "The process uses the trapped solar energy from inside the plants to volatilize the lead material. This condenses on a condensing plate of lead oxide that is then placed in a lead smelter."

However, Cunningham warns that the weeds' lead accumulation abilities are not consistent across soils. Most metals, and lead in particular, have numerous forms in the soil, not all of which are equally available for plant uptake, he says.

Plants that are tolerant to heavy metals are relatively common but most do not accumulate significant quantities of the metals. Cunningham began his search for plants suitable for phytoremediation with a small group of plants called hyperaccumulators. Often found growing on ore outcroppings, these plants showed what "biomining" could achieve. Nickel in their sap could reach over 25 per cent by dry weight, and lead levels were as high as 8,200ppm. But because they were small and grew slowly, they were not suitable for large-scale use, although breeding and molecular biology may change that in the future, claims Cunningham.

Plants evolve in this way because it is to their advantage, Cunningham says, as heavy metals control pathogens and insects. He is continuing his quest for "really weird plants" because he wants one able to uptake 20,000 ppm. But he might have to use genetic engineering to get one, he says.

Still, Cunningham is convinced that phyto-remediation is viable when pollutants are near the surface, are relatively non-leachable, cover large surface areas and pose little immediate risk to health or the environment.

Economically, Cunningham claims the prospects look excellent unless the waste site has been covered with concrete and needs to be dug up before planting. The major cost is the annual harvest, so trees which could store the lead for a long time would be the best solution, he says. Phytoremediation is not ready for full scale pilot demonstrations yet, but he hopes it soon will be. (Source: *Chemistry & Industry*, 3 May 1993)

E. PATENTS AND INTELLECTUAL PROPERTY RIGHTS

Canadian Plant Breeders' Rights Act amended

On 4 March 1991, Canada became a party to the International Convention for the Protection of New Varieties of Plants (UPOV) as revised in 1978. The Plant Breeders' Rights Act came into effect in Canada on 6 November 1991 following the publication of Regulations for canola, chrysanthemums, potatoes, roses, soybeans and wheat.

Amended regulations came into effect on 10 March 1993 which include the following newly prescribed categories of plants now eligible for protection: oats, dianthus, poinsettia, strawberry, barley, flax, apple, alfalfa, bean, pea, potentilla, cherry, pear, african violet, yew, grapes, corn.

The existing Act and Regulations provided for time periods following sale within which a breeder is entitled to apply for plant breeder's rights. The Regulations have been amended somewhat in this respect, and also transitional provisions have been provided for the most recently prescribed categories, provided that the application for protection is filed by 10 March 1994.

For applications filed until that date for one of the recently prescribed categories, the following time periods apply. A breeder of a new variety of apple, cherry, pear, yew or grapes is entitled to apply for plant breeders' rights provided he has not sold or concurred in the sale of the variety outside Canada prior to 1 August 1984 (new Regulation 7 (1)(a)) or inside Canada prior to 1 August 1990 (old Regulation 6). For a new variety of any of the other recently prescribed categories, a breeder is entitled to apply for plant breeders' rights provided he has not sold or concurred in the sale of the variey outside Canada prior to 1 August 1986 (new Regulation 7(1)(b)) or inside Canada prior to 1 August 1990 (old Regulation 6).

For a new variety of one of the categories of plants formerly prescribed, and for new varieties of all categories of plants for which applications are filed after the transitional provisions expire on 10 March 1994, the following time periods apply. The Canadian application or a foreign priority application must be filed before the breeder sells or concurs in the sale of the new variety in Canada. With respect to sales or concurrence in the sale of a new variety outside Canada, the breeder has six years from such activity within which to file its Canadian application for a new variety of apple, cherry, pear, yew or grapes, and four years from such activity within which to file its application for any other new variety. (Source: *The AgBiotech Bulletin*, Vol. 1, No. 3, May/June 1993)

Erythropoietin six-year patent dispute settled

After almost six years in and out of courts, two biotechnology firms - Amgen and Genetics Institute - have settled their bitter dispute over patent rights to erythropoietin (EPO).

EPO, used to stimulate red blood cell growth in anaemic patients, is the biotech industry's leading drug. Amgen's US sales for dialysis patients alone reached $506 million in 1992. Sales are also growing abroad and for other uses. Genetics Institute cannot sell EPO in the US, but markets it abroad.

Amgen has been in a stronger position since 1991, when a federal appeals court upheld its principal EPO patent and ruled Genetic Institute's patent invalid. After unsuccessful appeals which went as high as the US Supreme Court, Genetic Institute has agreed to settle, and will pay Amgen $14 million.

In early 1990 - while both companies' EPO patents still were considered valid - a federal court ordered the two firms to reach a cross-licensing agreement. However, no agreement was reached and the fight continued.

The settlement clears the way for Amgen to receive additional patents. In late 1991, the Patent & Trademark Office decided a patent interference proceeding in Amgen's favour. But Genetics Institute made a last-ditch attempt to challenge the ruling. All lawsuits have now been dropped, and Amgen expects to receive process and product patents for recombinant EPO. (Extracted from *Chemical & Engineering News*, 17 May 1993, p. 17)

Burroughs Wellcome AZT patents upheld in court

In a move that put a premature end to a jury trial, a federal judge has decided to uphold Burroughs Wellcome Co.'s patent rights to the drug AZT (zidovudine), used to fight human immunodeficiency virus (HIV) infection and AIDS.

The ruling, made on 22 July 1993 in US District Court in New Bern, NC, is against efforts by two smaller drug firms - Barr Laboratories (Pomona, NY) and Novopharm (Schaumburg, IL) - to offer cheaper, generic forms of the drug. Barr and Novopharm have appealed the decision to the US Court of Appeals in Washington, D.C.

The case stemmed from patent infringement complaints filed by Burroughs Wellcome against Barr and Novopharm, which are seeking rights to market generic forms of AZT and have filed abbreviated new drug applications to that effect with the Food & Drug Administration. The two generic drugmakers contend that researchers at the National Institutes of Health are co-inventors of AZT and that NIH should therefore have some rights to the drug's patents - which are currently held solely by Burroughs Wellcome and do not expire until 2005.

NIH supports the companies in this view, and is willing to let them produce generic AZT. The agency has already granted Barr a non-exclusive licence to market AZT. Novopharm also has applied to NIH for a licence, but the agency has delayed granting it pending resolution of the patent-infringement case. NIH also could have sued Burroughs Wellcome directly, but has held off doing so, in part because it believes the patent validity issue is being adequately explored in the private-sector lawsuit.

The pending appeal of the case will seek to obtain a judgement on whether the ruling was proper. If the appeals court agrees with the defendants' claims that the judge's decision was inappropriate, the case would probably be remanded back to the same district court, with a new jury but probably not with the same judge. (Abstracted with permission from *Chemical & Engineering News*, 9 August 1993, p. 14. Copyright (1993) American Chemical Society)

Researcher donates patent rights to WHO

Manuel Patarroyo, the Colombian immunologist who claims to have produced a malaria vaccine, is a man of his word. More than three years ago-as sceptical researchers attacked the methodology and ethics of his field trials-Patarroyo pledged that if the vaccine proved effective, he would donate his patent rights to the United Nations.

Patarroyo published positive results from a new Colombian clinical trial in the *Lancet* early in 1993. In May 1993 he signed over the rights to the World Health Organization's Tropical Diseases Research (TDR) programme.

If the vaccine's promise is confirmed, he wants it made widely available as quickly as possible, and not tied up in lengthy discussions about royalty payments. Furthermore, he says, he would not feel comfortable making money from the vaccine for himself or for his Institute of Immunology at the National University of Colombia in Bogota, since his work relied on some 31,000 Colombians who took part in clinical trials. (Extracted from *Science*, Vol. 260, 28 May 1993)

F. BIOINFORMATICS

Asia-Pacific Journal of Molecular Biology and Biotechnology

In the drive to be the major growth region into the third millennia, biotechnology is going to play an important role in many of the economies of the Asia-Pacific region. As the conceptual and scientific basis of modern biotechnology, molecular biology is having a powerful impact on many of the research problems of concern to the countries in the Asia-Pacific region. The application of the various approaches associated with recombinant DNA technology to various research problems is beginning to bear fruit. However, within the region, a vehicle for the publication and dissemination of research results is not as yet available. Regional researchers have little choice but to submit the results of their work to journals in the "developed" countries, often encountering biases associated with their research being perceived as "lower priority" to readers in the developed nations.

It thus seems appropriate and timely that the initiative be taken to publish a regional journal in the Asia-Pacific region to cater to this need in the field of molecular and cellular biology, and biotechnology. As a result of rapid progress in the past few years, a core group of researchers is now available in the region to form a credible and effective editorial team. The editorial team which has been assembled spans all the basic and applied aspects of molecular biology and biotechnology and is distinctly Asia-Pacific in its representation.

The Journal will be a joint publication of the Molecular Biology/Genetic Engineering Subprogramme of the National Working Group on Biotechnology, National Council for Scientific Research and Development (MPKSN), Ministry of Science, Technology and Environment, Malaysia and the Malaysian Society for Molecular Biology and Biotechnology. Although the publication of the Journal is based in Malaysia, the primary objective of the Journal is the promotion of molecular biology and biotechnology in the Asia-Pacific region and, ultimately, to be accepted globally as an important scientific publication.

The Journal will be committed to the advancement and dissemination of fundamental knowledge in the fields of molecular (and cellular) biology and biotechnology. Papers are solicited from researchers in all relevant areas of basic and applied research, including medicine and human health, veterinary medicine, agriculture, industrial, food and environmental biotechnology. Of special interest will be papers related to research relevant to the needs and problems of the Asia-Pacific region.

The Journal will publish the following types of papers: (1) Research papers; (2) Research notes (brief reports); (3) Occasional mini reviews (invited); (4) Book reviews; (5) Selected abstracts from regional scientific meetings; (6) Diary of scientific events in the region.

Change in MSDN host computer results in improved services

The Microbial Strain Data Network (MSDN) is an international non-profit-making organization providing specialized information and communication services for life scientists. The MSDN is run from a secretariat based in Cambridge, UK, with part-time support from the US, and overseen by an international Committee of Management. MSDN is especially concerned with the promotion and establishment of communication links world-wide in order to encourage scientific data exchange and cooperation.

A unique package of specialized on-line databases and bulletin boards are distributed through the MSDN network covering microbiology, biotechnology, genetics and biodiversity information. Diverse data sources include information on microbial isolates characterized in culture collections world-wide, bacteria and virus taxonomic information, bibliographic and legislative information, and others. Databases describe hybridomas, cell lines, and molecular probes as well as micro-organisms. MSDN subscribers additionally have access to the databases distributed by IRRO (Information Resource on Release of Organisms into the Environment).

As of 1 May 1993 MSDN moved its host computer from British Telecom to the Base de Dados Tropical (BDT) computer in Brazil. This is the first stage in the development of MSDN as a distributed system of regional nodes. The move takes advantage of the latest networking technologies and provides a much improved service for MSDN subscribers. MSDN is now an integral part of the Internet and is easier and cheaper to use than previously. The change of host has resulted in several significant changes as regards the on-line resources now available, and all directly benefit subscribers of the MSDN network.

The host computer can be accessed in seconds from most regions in the world. The MSDN network continues to be available through the public data networks (X.25, IPSS, GNS). The new computer address (NUA) is 72411925019. For subscribers in the US and Canada access is possible through Econet via SprintNet.

A major development is that MSDN is now directly accessible through the Internet (bdt.ftpt.br or 192.207.195.1). The Internet is a huge global network of computers originating from the academic and research communities, and now increasingly used by commercial organizations. Universities and other academic establishments are likely to have an Internet connection giving the benefit of free telecommunication links to MSDN.

For those currently without access to the Internet, and who are interested in this access path, there are a number of third party Internet service providers who enable commercial users to use the Internet. Examples include Delphi (US), Demon (UK), and EUnet (several European countries). The MSDN secretariat staff will be happy to provide further details and advise on access.

A completely revised method of payment has been introduced allowing unrestricted use of MSDN databases. A flat subscription fee is requested in advance on registration and covers a six monthly period. It is no longer necessary to watch the clock when searching, as charges are not based on connect time. An exception is Biotech Knowledge Sources (BKS) where a database surcharge is applied.

Charges are as follows:

	UK pounds	US dollars
Individual (single mailbox)	75	95
Group (up to six mailboxes)	350	495
Institution (up to 20 mailboxes)	700	995

An incredible variety of Internet hosted genetic, cell biology, ecology and other scientific resources provide a valuable complement to the MSDN databases. These include molecular sequence data banks, cell line and molecular probe databases, specialist scientific news and discussion groups (e.g. on biological species conservation and diversity), natural products database, and many more.

Most MSDN distributed databases now use a common search software, INFO, developed by BDT. This is a simple to use yet powerful text retrieval system. A series of menus allows browsing through the databases and bulletin boards. If preferred a particular database can be selected directly.

A new feature is the facility to carry out cross-file searches. If unsure of the best database to use, a search can be made of all database indexes. The number of records satisfying the search is listed for each database enabling the most appropriate database(s) to be selected.

A directory of MSDN subscribers is searchable on-line. On registration, subscribers are assigned an MSDN ID and password. For those on the Internet, electronic mail sent to MSDN mailboxes is forwarded to their Internet address. For those not on the Internet the MSDN ID can be quoted as an Internet mailbox address allowing easy Email exchange between Internet and non-Internet users.

In order to register with MSDN contact either:

MSDN Secretariat, 307 Huntington Road,
Cambridge CB3 0JX, UK
Tel.: 44-223-276622 Fax: 44-223-277605
Email: lynn@phx.cam.ac.uk or msdn@bdt.ftpt.br

Or

Bioinformatics Department, ATCC
12301 Parklawn Drive
Rockville, MD 20852, USA
Tel.: 1-301-231-5585 Fax: 1-301-770-1541
Email: lynn@atcc.nih.gov

The two full-time MSDN staff members based at the secretariat have over 16 years of combined experience in on-line information systems especially covering the biotech field. On demand, MSDN offers these skills through its information brokering and training services. If any individual or organization has a need for a comprehensive search to be undertaken, MSDN will use the most appropriate on-line hosts to retrieve the desired information. Customized searches can be made on a one-off or regular basis. Similarly, training and consultancy in microbial information management and retrieval can be provided to individuals or groups. Charges will be quoted on request.

Bio boost

Cambridge has won the close-run race to host the European Bioinformatics Institute, giving a boost to the city's reputation as a centre of excellence in molecular biology.

The EBI will provide a database of information on genomes to scientists across Europe and joins seven other large molecular biology centres in Cambridge. The city fought off bids from Sweden and Germany to host the centre. (Source: *New Scientist*, 20 March 1993)

EC BioRep update

The BioRep database is a "Permanent Inventory of Biotechnology Research Projects in the European Communities". It was begun in 1987 and is sponsored by the EC and managed by the Library of the Royal Netherlands Academy of Arts and Crafts. It contains information on almost 5,000 biotechnology projects in progress in 1,664 European institutes. BioRep is used increasingly by EC researchers to find research partners, and by industry to find research expertise and is available on-line on the ECHO host.

New biotech directory

A third edition of the *Australian and New Zealand Biotechnology Directory* has just been released. Published by the ABA, biotech's industry association, it is packed with over 300 pages of information on biotechnology companies, research organizations and activities.

It is well produced and thoroughly indexed and cross-referenced. It is an excellent source of information on Australian biotechnology at $A60.00 plus postage. It is available from the ABA (P.O. Box 4, Gardenvale, 3185) Tel 03-596-8879, Fax: 03-596-8874.

China's Biotech Directory

Han Communications have released an English language biotechnology directory for mainland China. The publication is around 250 pages and the cost is US$199.00. Han Communications address is: PO Box 71006, Wuhan, Hubei 430071, PR China (Fax: +86 27 718343). Publisher: Canadian Biotechnology News Service, 20 Stonepark Lane, Nepean, Ontario K2H9P4 (Fax: 613-726-7344).

Biotechnology Today, Performance and Prospects to 2000

This latest report provides an overview of all the main biotechnology sectors. Analysis of the major health-care and agriculture markets, as well as the growing potential of food processing, detergents, energy and environmental applications.

Coverage centres on:

- Progress in the main sectors in which novel biological processes are used
- The biotech industry's current financial strategies
- Intellectual property rights and protection
- The political environment for biotechnology in each of the main markets
- Key projections showing the industry's growth up to the year 2000.

Biotechnology Today helps meet both the immediate and long-term needs of any professional involved with the industry. It has been written to be accessible to the non-technical executive. To bring today's business opportunities into focus.

The report is only available direct from the Financial Times at US$372 with your credit card details on 0209 612493. Further information from Helen Nicol, Marketing Manager, Financial Times Management Reports, Editorial and Marketing, 7th Floor, 50-64 Broadway, London SW1H 0DB. Tel.: +44(0)71-799-2002; Fax: +44(0)71-799-2259.

Essays on biodiversity

Vandana Shiva, *Monocultures of the Mind Biodiversity, Biotechnology and the Third World* Published by Third World Network, 87 Cantonment Road, 10250 Penang, Malaysia, 1993, ISBN 983-9747-04-5, 184 pp.

This latest publication by the famous Indian woman activist and environmentalist is a collection of five essays in which she brings together her thinking on the protection of biodiversity, the implications of biotechnology, and the

consequences for agriculture of the global pre-eminence of Western-style scientific knowledge. The author examines the current threats to the planet's biodiversity and the environmental and human consequences of its erosion and replacement by monocultural production. She shows how the new Biodiversity Convention adopted at the Rio Earth Summit has been gravely undermined by a mixture of diplomatic dilution during the process of negotiation and Northern high-tech interests making money out of biotechnologies. She explains what these technologies involve and gives examples of their impact in practice. She questions their claims to improving natural species for the good of all and highlights the ethical and environmental problems posed. Her major criticism goes against the North's particular approach to scientific understanding which has led to a system of monoculture in agriculture - a model that is now being foisted on the South, displacing its societies' ecologically sounder, indigenous and age-old experiences of truly sustainable food cultivation, forest management and animal husbandry. This rapidly accelerating process of technology and system transfer is impoverishing huge numbers of people, disrupting the social systems that provide them with security and dignity.

The author calls for a halt, at international as well as local levels, to aid and market incentives to both large-scale destruction of habitats where biodiversity thrives and the introduction of centralized, homogeneous systems of cultivation.

Agricultural biotechnology in developing countries: a cross country review by John Komen and Gabrielle Persley. ISNAR. 45 pages.

The International Service for National Agricultural Research (ISNAR), with funding from the World Bank and the Governments of Australia and the Netherlands, has undertaken a number of in-depth studies of biotechnology in selected developing countries. ISNAR also conducted a four-year study (1988-1992) entitled "Agricultural Biotechnology: Opportunities for International Development".

The present report is the first of a series, planned by the Intermediary Biotechnology Service (IBS) whose establishment resulted from a recommendation by the Biotechnology Task Force (BIOTASK) of the Consultation Group on International Agricultural Research (CGIAR). IBS offers a demand-driven, problem-oriented advisory service to make available the expertise of advanced biotechnology institutes to the developing countries.

Developing countries are investing in infrastructure and human resources to support national biotechnology programmes and adopting policies to facilitate biotechnology R&D in both the public and the private sectors.

This reports provides a short, well-organized and informative comparative description of the different approaches taken by the governments of the following 10 countries: China, Colombia, Egypt, India, Indonesia, Kenya, Malaysia, the Philippines, Thailand and Zimbabwe. Between them, they include half the world's population.

The report analyses the institutional organization adopted in the various countries and describes how governments address the issues constraining further development of biotechnology. Major conditions identified for productive programmes include:

- Close collaboration between new biotechnology and conventional agricultural research (especially plant breeding) to ensure that new techniques are taken through to new products and field application;
- Minimal duplication of expensive equipment and services;
- An effective working environment for well-trained scientists and adequate financial resources.

Possible institutional arrangements include:

- Establishing a national biotechnology agency to coordinate and fund biotechnology within existing institutions and to determine national policies;
- Stimulating research at designated centres of excellence;
- Creating a national biotechnology institute.

The report discusses the advantages and disadvantages in the context of specific countries and emphasizes the importance of private sector involvement and finance.

For information about IBS and availability of this succinct and valuable report, contact:

Dr. Joel Cohen, Project Manager, IBS, ISNAR,
P.O. Box 93373, 2509 AJ The Hague,
The Netherlands
Tel.: (31)70-349-6100; Fax: (31)70-381-9677.

Cultivating Knowledge: Genetic Diversity, Farmer Experimentation and Crop Research; Walter de Boef, Kojo Amanor and Kate Wellard, with Anthony Bebbington

This book contains up-to-date information on current efforts of conservation and development of local crop diversity and the important role of farming communities in this respect. It reflects new approaches and concepts in the field of conservation, enhancement and utilization of local crops, with case studies from Africa, Latin America and Asia.

The book also looks at policy issues raised by the expansion of agribusiness, and the need to consider the interests of small-scale farmers. The authors come from a variety of backgrounds, from plant breeders to anthropologists, international researchers to NGO development workers and lobbyists, and present a number of different views and perspectives on the subject. (Available from Intermediate Technology Development Group, 103/105 Southampton Row, London WC1B 4HH, UK).

Green Globe Yearbook of International Cooperation on Environment and Development, 1994. Editors: Helge Ole Bergesen and Georg Parmann. Publisher: Oxford University Press, Walton

The *Green Globe Yearbook* is an independent publication on the environment and development from the Fridtjof Nansen Institute in Norway which specializes in studies of international resource management.

The *Yearbook* consists of two separate parts: analysis and reference. The former comprises 11 articles written by independent experts with in-depth knowledge of a particular issue. The 1994 edition covers the international dimensions of deep seabed mining, oil spills at sea, illegal timber trade, the problem of migratory species, desertification, nuclear reactor safety, transfer of environmentally sound technology, follow-up of Agenda 21, and international attitudes toward environment and development, as well as exploring the role of environmental treaty secretariats and the new Commission on Sustainable Development.

The reference section contains systematically listed key data concerning the most important international agreements on environment and development, and inter- and non-governmental organizations with activities in this area.

The combination of independent, high-quality analysis and a useful reference section makes this *Yearbook* unique in the dissemination of environmental information. It is an indispensable guide for decision makers in government, international organizations, NGOs, and industry as well as an essential source book for students, policy makers, and the public at large. (More information available from: The Fridtjof Nansen Institute, Fridtjof Nansens vei 17, P.O. Box 326, N-1324 Lysaker, Norway. Fax: +47 67-125047, E-mail: iliseter@ulrik.uio.no.)

The Dictionary of Biological Control and Integrated Pest Management

The Dictionary of Biological Control and Integrated Pest Management is an essential reference for researchers, manufacturers and growers who need to make sense of the whole new vocabulary of agricultural and biotechnological jargon that has arisen as a result of the rapid commercial development of predators and parasites, micro-organisms and methods for the non-chemical control of pests.

This is claimed to be the first *Dictionary* covering the biological control field - an invaluable publication for anyone working or studying in this field.

Aspects of biological control and integrated pest management covered by the *Dictionary* include: processes, research, technology, taxonomy, bacteria, fungi, viruses, nematodes, predators, parasites, pheromones and diseases. Further information from: CPL Scientific Ltd., Science House, Winchcombe Rd., Newbury, Berks, RG14 5QX, UK. Tel.: +44(0)635-524064; Fax: +44(0)635-529322.

The Worldwide Directory of Agrobiologicals 1993/94 - Third Edition

The Worldwide Directory of Agrobiologicals has become the essential source of product information for manufacturers, distributors, farmers, growers and government agencies in over 42 countries from the United States and Western Europe to Indonesia, Taiwan and Mongolia. The third edition contains over 1,000 revised product listings and updates on 276 active ingredients and 326 companies. A new product section finder and indexes of products by company and by active ingredient make it quicker and easier than ever before for you to find the information you need.

Contents: reports on 132 companies; introduction to agrobiologicals (*Bt*, nematodes, fungi, insects, pheromones); registration and legislation including The EC Pesticide Directive; products - insect control, fungal and disease control, weed control, silage inoculants, probiotics for animals, rhizobium, fertilizers, compost and soil activators, others; appendices - product section finder, active ingredients (276) and product names index, companies (268) and product names index, company addresses and contacts (326).

Further information from: CPL Scientific Ltd., Science House, Winchcombe Rd., Newbury, Berks, RG14 5QX, UK. Tel.: +44(0)635-524064 Fax: +44(0)635-529322.

Harmful non-indigenous species in the United States

Much energy has been expended over the last decade debating the issue of whether environmental releases of genetically engineered organisms should be compared to agricultural breeding or to the release of exotic organisms, like the multiflora rose, purple loosestrife or kudzu.

Although the details of the debate quickly become complex, put simply, considering releases of engineered organisms to resemble agricultural breeding leads to predictions of lower risk than a comparison to exotic organisms.

A recent report from the Office of Technology Assessment (OTA) (*Harmful Non-Indigenous Species in the United States*), released in October 1993, offers a new framework for that debate. Rather than contrasting the two, the OTA encompasses both agricultural breeding and exotic releases under a more comprehensive category, non-indigenous species (NIS). The report includes all genetically engineered organisms as NIS.

According to the report, NIS are organisms living beyond the geographic area that they would inhabit if they were not affected by significant human influence. In addition to genetically engineered organisms, the non-indigenous designation covers all domesticated and feral species. Feral plants and animals are those free-living plants or animals whose ancestors were once domesticated. An example would be Johnson grass, a plant once grown for forage but now a tenacious weed. Also included as NIS are hybrids except those formed naturally between indigenous species. Thus all hybrids of non-indigenous crops like corn and wheat are NIS; many hybrids of even an indigenous crop like blueberries would be considered NIS because they are products of human selection.

The report acknowledges that most NIS are beneficial. US agriculture depends on non-indigenous crops and animals. In fact, most of the crops and livestock now grown in this country are native to other parts of the world. Examples are apple, beans, cattle, and cotton. In addition, other commercial enterprises such as sport fishing and hunting, aquaculture, and the pet business also rely on NIS.

But the OTA report also points out that many NIS have been harmful, causing problems ranging from nuisances to disasters. Some NIS like the imported fire ant and African honey bee threaten human health. Others like the Mediterranean fruit fly and boll weevil cause millions of dollars in crop losses every year. Still others like purple loosestrife disrupt ecosystems and displace native organisms. Many of the harmful NIS were originally introduced purposefully. These include multiflora rose and kudzu for erosion control, grass carp for weed control, and crabgrass for a grain crop.

Although the harmful NIS are a relatively small portion of the total, they affect all regions of the country, and the damage they do is substantial. Control costs, where control is an option, run into the billions of dollars.

The OTA report recasts rather than resolves the debate between the agricultural and the exotic models. It puts agriculture into the proper context as a source of both beneficial and harmful NIS, gives new urgency to the protection of indigenous flora and fauna, and makes it clear that genetically engineered organisms are among the many kinds of foreign species that pose environmental threats.

The OTA report (OTA-F-565) is available for $21 from New Orders, Superintendent of Documents, P.O. Box 371954, Pittburgh, PA 15250-7954, Tel: (202)783-3238.

Africa in looseleaf

Everything there is to know about maize varieties, potato diseases, artificial fertilization of vanilla or the preparation of cloves, it's all easy with the *Fiches techniques d'agriculture spéciale à l'usage de l'enseignement agricole en Afrique subsaharienne*. Aimed specifically at teacher and students, these abundantly illustrated didactic looseleaf sheets are published by the French Bureau d'études Spécialisé en Développement Rural (BDPA-SCETAGRI), with the collaboration of the Agridoc international network and the support of the French Ministère de la Coopération. Each crop is examined under the headings of utilization, production and consumption statistics, botany, and techniques of cultivation, transformation and processing. The first lot of four dossiers, released in 1992, includes maize, cloves, vanilla and potatoes. A second lot published in 1993 deals with banana, cacao, coconut and manioc (cassava). Other packages are planned covering yam, pear, soya, sorghum, peanut (groundnut), mango, sweet potato and irrigated rice. Available in French from: BDPA-SCETAGRI, Centre de documentation et d'information, 27 rue Louis Vicat, 75738 Paris Cedex 15, France. Tel.: 33(1)4638-3475; Fax: 33(1)4644-7544. Price: 110 FF for four packets.

Wine microbiology and biotechnology

This book, edited by University of New South Wales wine expert Dr. Graham Fleet, draws on the expertise of an international list of authors. The book contains 17 chapters. Each chapter is written as a review, covering examples of the latest research, and with some speculation on future trends. It is truly international in content, with Australian, US, German, French, Swiss, Japanese, Canadian and Italian authors. It covers in a comprehensive and up-to-the-minute manner the current views on the chemistry and microbiology of wine-making. As an example, Doris Rauhut's chapter on the production of sulphur compounds by yeasts is a fascinating, but often neglected, area strongly involved in imparting flavour to wine. This chapter is complemented by a chapter on sulphur dioxide and the effects of its addition to wine quality and preservation by the Italian authors, Romano and Suzzi.

Overall, the book is an ideal teaching text and one of the few up-to-date volumes available. It is clearly printed, well bound, and the figures and tables are clear. The book is well written, easy to read and thoroughly referenced.

Editor: G.H. Fleet ISBN 3-7186-5132-7, hardcover, 520 pages, US$ 120, December 1992. (Harwood Academic Publishers, Switzerland)

G. SPECIAL ARTICLE

BIOTECHNOLOGY FOR CASH CROPS OF DEVELOPING COUNTRIES: OPPORTUNITIES, PROSPECTS AND THREATS

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Introduction

Agriculture is very important to the economies of most of the developing countries (DCs) of the world and in some instances, constitutes their main economic activity. Although there is a need for ever-increasing food production in these countries, the production of cash crops continues to be relevant for the generation of vital export earnings, economic growth and employment creation (18).

The major cash crops of DCs are generally those that are produced largely or exclusively for export and include coffee, cocoa, palm oil, rubber, sugarcane and tea. However, several other crops have assumed importance as exports from various DCs. They include avocado, banana, cotton, various nuts (e.g. cashew and pistachio), coconut, citrus, date palm, fresh vegetables, groundnuts, jute, litchi, mango, pineapple, pyrethrum, spices (cardamon, pepper, pimento, vanilla), ornamental flowers, papaya, soyabean, sisal, sunflower, tobacco, and timber (1, 7, 12).

Prior to 1950, the DCs had significant research programmes for the improvement of the so-called "colonial trade crops" such as sugarcane, tea, coffee, cocoa, cotton and rubber (11). However, after independence, food crops were accorded greater attention although research on cash crops has continued to varying degrees due to their economic importance. While significant advances in the production of cash crops have been achieved through the use of conventional breeding and the adoption of improved agronomic practices, several constraints still limit increased and sustainable production. Biotechnology offers new and exciting opportunities, particularly for the amelioration of important constraints which have proved intractable to conventional techniques (9, 13, 15, 21, 28, 30, 34). It must, however, be viewed as a supplementary tool rather than a substitute for conventional approaches to improved crop production.

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The potential role of biotechnology includes the following:

- Production of disease-free planting material;
- Rapid propagation of superior genotypes;
- Reduction of breeding time;
- Development of transgenic plants with resistance or tolerance to important diseases, pests and abiotic stresses;
- Transfer and storage of germplasm;
- Genome mapping to facilitate selection;
- Development of herbicide-tolerant transgenic plants which may allow for more effective and widespread weed control;
- Development of diagnostics for pathogen detection.

The state-of-the-art in biotechnology for important cash crops is shown in table 1; its possible applications and suitability for the amelioration of major constraints are illustrated in table 2.

Production of disease-free planting material

Insidious and debilitating systemic infections by viruses, viroids, fungi, bacteria and mycoplasma-like organisms (MLOs) afflict several crops in DCs and are particularly serious in those that are vegetatively propagated. Nematode infestations also pose problems in some crops such as bananas. Unchecked, these infections pose a serious threat, particularly for the majority of DC farmers who continually produce their own planting material. Biotechnology offers an appropriate and simple solution to this problem through the development of commercial-scale *in vitro* techniques for the elimination of systemic infections and the production of pathogen-free planting material (25). Shoot- and meristem-tip culture have proved successful in banana, date palm, pineapple and rubber but continue to be problematic for cocoa, coffee, coconut, oil palm and pepper (2, 5, 25, 27, 30, 42). In citrus, shoot-tip grafting and polyembryonic seed production has obviated the need for meristem- and shoot-tip culture (30). Regeneration through organogenesis or somatic or zygotic embryogenesis offers considerable prospects for several crops if it can be developed to a commercial scale (34). In general, the development and application of *in vitro* technology for pathogen-elimination is within the reach of most DCs and could have enormous implications for crop production.

Table 1

Biotechnology state-of-the-art for major cash crops produced in developing countries

Crop	Micro-propagation and disease elimination	Regeneration	Transformation	Potentially useful transgenics	Mapping
Avocado	+	-	-	-	-
Banana	+++	+	+	-	+
Cardamom	+	-	-	-	-
Citrus	+++	-	-	-	-
Cocoa	+	-	-	-	-
Coconut	+	+	-	-	-
Coffee	++	+	-	-	-
Cotton	na	+	+	+	+
Date palm	+	++	-	-	-
Groundnuts	na	+	+	-	+
Mango	+	+	-	-	-
Oil palm	+++	++	-	-	-
Pepper	+	-	-	-	-
Pineapple	+	+	-	-	-
Pyrethrum	++	-	-	-	-
Rubber	++	+	-	-	+
Soyabean	na	++	++	+	+
Strawberry	++	+	+	-	-
Sugarcane	++	-	+	-	-
Sunflower	na	+	+	-	-
Tea	+	-	-	-	-
Tobacco	na	+++	+++	+	+
Trees (forestry)	+	+	+	-	
Vanilla	+	-	-	-	-

Codes: na, not applicable; -, not developed; + just beginning; ++ widely used; +++ routine

Table 2

Major constraints, conventional solutions, potential application and suitability of biotechnology solutions

Crop	Constraint	Conventional solution	Biotechnology solution	Suitability
Avocado	Black spot	heat treatment	not available	-
Banana	Propagation	vegetative shoots	micropropagation	++
	Black Sigatoka	breeding; chemicals	not available	++
	Panama disease	breeding; chemicals	not available	++
	Fusarium wilt	breeding	not available	++
	Nematodes	nematicides	micropropagation	++
	Viruses	none	micropropagation, CPMP	+
Citrus	Propagation	grafting, seeds	shoot-tip grafting	-
	Viruses	polyembryonic seeds	shoot-tip grafting	+
Cocoa	Propagation	seeds; rooted cuttings	micropropagation	++
	Viruses	not available	micropropagation	++
	Moniliasis	breeding; chemicals	not available	+
	Black pod	chemicals	not available	-
	Canker	chemicals	not available	-
	Witches broom	chemicals	not available	++
Coconut	Propagation	seed	not available	+
	Lethal yellowing	breeding	diagnostics	++
Coffee	Propagation	seed, vegetative	micropropagation	++
	Berry disease	breeding	not available	++
	Rust	breeding	not available	+
Cotton	Verticillium wilt	breeding	not available	+
	Bacterial blight	breeding	not available	-
	Insect pests	chemicals	Bt and CpTI transgenics	++
Date palm	Propagation	seed, vegetative	micropropagation	++
	Tracheomycosis	not available	micropropagation	++
Groundnuts	Viruses	not available	CPMP	++
	Leaf spots	breeding	not available	-
	Rust	breeding	not available	-
Mango	Propagation	vegetative buds	not available	-
Oil palm	Propagation	seed	micropropagation	++
Pepper	Viruses	not available	micropropagation; CPMP	++
Pineapple	Propagation	vegetative suckers	micropropagation	++
Pyrethrum	Propagation	vegetative	micropropagation	++
Rubber	Propagation	seed, vegetative buds	micropropagation	++
	Leaf blight	breeding	not available	+

Table 2. (continued)

Major constraints, conventional solutions, potential application and suitability of biotechnology solutions

Crop	Constraint	Conventional solution	Biotechnology solution	Suitability
Soyabean	Nitrogen	fertilizer	improved inoculant	++
	Web blotch	breeding	not available	-
	Insect pests	chemicals	Bt transgenics	+
Sugarcane	Propagation	vegetative cuttings	not available	-
	Loose smut	breeding; chemicals	not available	-
	Fiji disease	breeding; chemicals	somaclonal variants	+
Sunflower	Leaf spots	breeding	not available	-
	Sclerotinia	breeding	not available	-
Tea	Propagation	stem cuttings	micropropagation	+
	Root rots	chemicals	not available	-
Tobacco	Virus diseases	breeding	CPMP	+
	Alternaria blight	breeding; chemicals	not available	-
	Wildfire	breeding; chemicals	BSP transgenics	+
	Blue mould	breeding; chemicals	not available	+
	Nematodes	breeding; chemicals	not available	+
	Insect pests	chemicals	Bt and CpTI transgenics	+
Trees	Propagation	seeds, vegetative	micropropagation	++
Vanilla	Propagation	stem cuttings	micropropagation	+

Suitability: -, not suitable; +, suitable; ++, highly suitable; BSP: Bacterial self-protection; CPMP: virus coat protein-mediated protection; Bt: Bacillus thuringiensis; CpTI: Cowpea trypsin inhibitor

Rapid propagation of superior genotypes

Micropropagation of elite and pathogen-free genotypes is most applicable to species that are either perennial, sterile, outcrossed and therefore highly heterozygous, or suffer from persistent systemic infections. A variety of techniques including meristem- and shoot-tip culture, enhanced precocious axillary shoot formation and culture, organogenesis and somatic embryogenesis have been developed for banana, cardamon, chrysanthemum, date palm, oil palm, pineapple, pyrethrum and rubber (25, 27, 30, 34). While significant progress in micropropagation has been made in other crops such as cocoa, coconut, coffee, mango, pepper and some trees (2, 19, 30, 34), commercial-scale operations for these crops are yet to be achieved. The use of a liquid cell-culture bioreactor system based on somatic embryogenesis is potentially the most promising and rapid technique but is still being developed for banana, pineapple, coffee and ornamental palms (31). Micropropagation, coupled with pathogen elimination where appropriate, offers immense prospects for increased and sustainable crop production in DCs. Its modest technical requirements and low cost relative to other biotechnological techniques make it particularly suitable and cost-effective. However, the clonal fidelity problems encountered in banana, coconut and oil palm (27, 30, 34) warrant careful consideration during the commercialisation of micropropagation systems.

Generation of genetic variation

The production of somaclonal variants may be useful for the development of novel genotypes with resistance to biotic and abiotic stresses, particularly if such traits cannot be found in germplasm collections and wild relatives. However, although potentially useful variants for disease resistance have been obtained in sugarcane, tobacco and other crops (31, 34), little progress has been made in

the development of novel genotypes for commercial production.

Transgenic resistance/tolerance to diseases, pests and abiotic stresses

Transformation with reporter or potentially useful genes has now been achieved in several crops (14, 15, 16, 20) and will probably become more widespread due to the use of biolistic techniques. However, the unavailability of efficient regeneration techniques will likely continue to be the main obstacle to the widespread use of recombinant DNA technology for crop improvement.

Transgenic plants carrying novel *Bacillus thuringiensis* (Bt) genes, proteinase inhibitors, viral coat-protein genes and bacterial self-protection (BSP) genes are likely to have a significant impact on pest and disease control, particularly within the context of reduced pesticide usage and sustainable agricultural production (4, 5, 6, 14, 15, 16, 20, 24). While the technology is yet to be commercialised in developed countries, it appears very promising although it should be noted that it is unlikely to be entirely problem-free. If commercially successful, and it can be transferred to, or developed in DCs, this technology could alleviate important pest and disease problems (table 2), reduce pesticide usage and also result in significant savings of foreign currency which would otherwise be used to procure pesticides from developed countries. Advances in the development of salinity-tolerant transgenic plants (33) are also of importance because abiotic stresses pose a major limitation to increased crop production in several DCs. However, DCs will have to wrestle with the problems of high cost, expertise demand, biosafety and proprietary protection if this technology is to be developed locally or transferred from developed countries. It is also important that DCs take cognisance of the Bt resistance "breakdown" problems that are looming in developed countries and attune themselves to the need for "resistance management" and rational implementation of transgenic technologies.

Reduction in breeding cycle

Long generation turnover time and slow propagation of improved genotypes pose problems in the breeding of coffee, cocoa, coconut, rubber and trees (1, 30, 34). Tissue culture techniques such as anther culture and micro-propagation offer viable solutions and significant progress has already been made in several crops (30, 34). Furthermore, biotechnology can expedite the genetic improvement of crops in which sexual incompatibility is a barrier to recombination and germplasm usage.

Germplasm storage

In-situ germplasm conservation of perennial crops can be very costly, demanding of space and risky due to exposure to disease. Tissue culture and cryopreservation offer possible solutions to these problems for crops such as banana, coffee, cocoa, oil palm, rubber, mango, avocado, pepper and sugarcane (36). However, although appropriate techniques have been developed for some of these crops, the feasibility and suitability of the technology remains questionable.

Genome mapping

The detailed mapping of plant genomes by Restriction Fragment Length Polymorphisms (RFLP) and Random Amplified Polymorphic DNA (RAPD) techniques is considered to hold enormous promise for the genetic improvement of various crops (32, 37). Potential practical applications of this technology include detection of important genes and improved selection for traits that are difficult or very demanding to score. Genes of interest would include those for disease resistance, quality attributes and quantitative traits such as yield. However, while the technology is fairly advanced for crops such as cowpeas, groundnuts, soyabeans and maize, very little progress has been made in the major cash crops of DCs. Although the technology is already being used in some breeding programmes in developed countries, it is yet to be become routine due to its high cost and expertise requirements. Furthermore, the validity of the technique for the manipulation of important quantitative trait loci (QTLs) has also been questioned (10). Hence, although it appears promising, it is as yet unproven for QTLs (10) and presently of limited value to DCs. Given that there are other more pressing problems, the benefits of genome mapping presently appear questionable and it is perhaps best left to developed countries which can afford it.

Herbicide tolerance

Genetic engineering of herbicide tolerance into crops such as maize, soyabean and tobacco ranks as one of the most important but highly controversial achievements of advanced biotechnology (17, 38). Although commercial production of transgenic plants with tolerance to more effective and supposedly environmentally-friendly herbicides is yet to become a reality, it is very likely that this technology will have major implications for high-input agriculture in developed countries. For DC's, the technology may be useful only for plantation crops or well-to-do farmers who normally use herbicides.

Diagnostics

Effective disease prevention and control is dependent upon precise identification of the causal organism. Although the diagnosis of most of the important diseases is not very difficult, some debilitating and sometimes unknown diseases continue to elude pathologists working in DC's. For viral diseases, the major limitations are inadequate training in virology, unavailability of ready-to-use test kits and the high cost of appropriate antisera. Even when test kits are available, strain differences may still hinder precise pathogen identification. Diagnosis of diseases caused by MLOs is more demanding although some progress is being made for important diseases such as lethal yellowing in coconut (3). In general, the ready

availability of improved diagnostics could make a significant contribution to the production of some cash crops, particularly if combined with pathogen-elimination techniques, micropropagation and sanitation.

Biotechnology threats

The threats posed by biotechnology to cash crop production include the following:

- Substitution of vanilla, pyrethrum, some oilseed crops, cocoa butter and sugarcane by the biotechnology-based products of developed countries (5, 21, 23, 26, 29);
- Marginalisation of resource-poor farmers and smaller producing countries who are unable to capitalise on biotechnology;
- Reduction in the biodiversity of crops due to widespread adoption of improved and rapidly propagated genotypes;
- Increase in the genetic vulnerability of crops to pests and diseases due to widespread dependence on limited protective measures such as Bt transgenics;
- Environmental degradation and negative impacts on sustainable agriculture due to more widespread and increased use of herbicides on transgenic herbicide-tolerant genotypes (17);
- Overproduction and consequently lower pricing due to rapid and widespread adoption of improved genotypes.

The most publicised negative effect of biotechnology relates to the substitution of sugarcane with high-fructose syrup (HFS) produced from maize (5). Possible substitution of vanilla, pyrethrum, cocoa butter and some oils is receiving increasing attention (5, 29) but is yet to become a reality. It is also argued that the worldwide shift to natural products coupled with their unique and complex characteristics will counteract substitution. Looming pest resistance to widely promoted Bt transgenics is of considerable concern (5). Therefore, as DCs ponder over the development, application and benefits of biotechnology, it is essential that they also consider and plan for possible negative impacts.

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MUSHNET Newsletter

International Network for Mushroom Biotechnology and Mushroom Bioconversion Science

Issue No. 2 | June 1994

Research and Training in Mushroom Biology at the Chinese University of Hong Kong

(Contributed by Professor S.T. Chang and Dr. J. A. Buswell of the Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong)

In October 1993, UNIDO approved the allocation of funding to establish a Centre for International Services to Mushroom Biotechnology (CISM BIOTECH) on the campus of The Chinese University of Hong Kong (CUHK). The Centre is currently engaged in developing a Mushroom Biotechnology Database and Information Network (MUSHNET) for the collection and dissemination of information relating to mushroom production and mushroom biotechnology. This will comprise of published literature relating to the subject areas, primary data available from CUHK records and through the World Society for Mushroom Biology and Mushroom Products, and other material available through national databases, government reports and other sources of a similar nature. A Mushroom Depository and Genebank for the conservation of mushroom genetic resources will also be created. The Centre will provide for technology transfer and services to organisations in developing countries through training courses, workshops and consultant activities, and undertake research in many aspects of mushroom biology including cultivation technology, genetics and biology of mushrooms, bioconversion of agricultural wastes, mushroom products, and processing and marketing. CISM BIOTECH will also interact with regional and national nodes/resource centres worldwide in providing the necessary technological back-up support for the promotion of regional and national development of mushroom and mushroom-related industries. The Chinese University of Hong Kong represents an ideal location for the Centre. The Department of Biology at the University has a long tradition of research and training in mushroom biology and is internationally recognised for its contributions to the subject area. At the present time, eight academic staff of the Department, a Visiting Professor from the United Kingdom (Professor John Peberdy), other visiting scientists and numerous graduate students are engaged on mushroom-related research projects. These include:

1. Development of Mushroom Nutraceuticals – a new class of compounds extractable from mushrooms which may be used in the prevention and treatment of various diseases and which can serve as dietary supplements to improve human health;

2. Enzyme production by edible mushrooms and the optimization of growth substrate utilization for increased mushroom yields;

3. Somatic hybridization of mushrooms by protoplast fusion;

4. Protoplast and molecular studies on *Volvariella volvacea;*

5. Construction of a genomic library for *V. volvacea;*

6. Influence of metals on the growth and metabolism of mushroom fungi;

Compiled by the Technology Service of UNIDO for the International Network for Mushroom Biotechnology and Mushroom Bioconversion Science. Further information available from the Technology Service, UNIDO, Vienna International Centre, P.O. Box 300, Vienna A-1400, Austria. Tel. (1)21131-0, Telex 135612, Cables: UNIDO VIENNA, Fax: 232156.

7. Electrophoretic karyotype analysis for the mushroom species *Coprinus cinereus*, *Pleurotus ostreatus*, *Schizophyllum commune* and *Lentinus edodes*.

8. Genomic library, linkage map and germplasm bank for *L. edodes*.

9. Hepatoprotective, anti-tumour and immunomodulatory activities of mushrooms.

10. Biological detoxification of rapeseed meal by edible fungi.

11. Identification and biological evaluation of glucosidase inhibitors from mushrooms.

12. Analysis of staphylococcal enterotoxin in canned mushrooms.

13. Development of mushroom-based processes for upgrading food industry wastes.

Since 1991, the Department has attracted almost US$ 850,000 in research funding for mushroom research from sources which include UNIDO, the Hong Kong Research Grants Council, the Croucher Foundation, the British Council and several industrial organizations. The Department of Biology at CUHK plays a major role in the training of young scientists, especially those from developing countries, in techniques related to mushroom biology and cultivation. In recent years, several workshops, financially supported by the United Nations Educational, Scientific and Cultural Organization (UNESCO), the United Nations Development Programme (UNDP) and the International Cell Research Organization (ICRO) have been held in the Department's research laboratories and at the mushroom cultivation facility located on the University campus. Moreover, the expanding worldwide interest in mushroom biology and mushroom biotechnology was clearly exemplified by the success of the First International Conference on Mushroom Biology and Mushroom Products held in Hong Kong in August 1993. The Conference, organised jointly by CUHK and the UNESCO Network for Microbial Resource Centres (MIRCENS), and hosted by the Chinese University of Hong Kong, attracted over 300 participants from 44 countries.

New Participant in MUSHNET

We have just received an enthusiastic application to participate in MUSHNET from India, the first so far. The application comes from Professor Suneela Marinkurve of the Department of Microbiology at Goa University. Professor Marinkurve writes that the State of Goa is very rich in its variety of mushrooms, which emerge with the onset of the monsoon. The Department's basic interest was to survey the area for wild edible mushrooms, which is compiled in the form of a doctoral thesis by Nandakumar Kamat. A summary of this work by Mr. Kamat is reproduced in this edition of the Newsletter.

Details from Professor Marinkurve are as follows:

1. Country: India

2. Name: Professor Suneela Mavinkurve

3. Organization: Goa University

4. Interests: Reseaarch and training – study of microfungi (a) Taxonomic identification of edible agarics species; (b) Germplasm bank from tissue culture from agarics characterization, preservation; (c) commercial cultivation of local varieties of mushrooms.

5. Contribute to MUSHNET: Detailed information as to the expected nature of contribution is requested.

6. Access to NET: IBM compatible computer available. Access to NET desirable.

7. Address: Department of Microbiology, Goa University, Taleigao Plateau, Goa – 403 203, India. Telephone: (0832) 223949; Fax No.: (0832) 224184 8. Position: Professor and Head, Department of Microbiology and Dean, Faculty of Life Sciences and Environment.

Wild Mushrooms in the State of Goa

(Summary of doctoral thesis by Nandakumar Kamat, Department of Microbiology, Goa University, India, contributed by Professor S. Mavinkurve)

About 70 per cent of the geographical area of the State of Goa was surveyed for wild agaricales (mushrooms), with emphasis on edibility, from 1986 to 1991. Altogether 73 field and market sites were sampled resulting in 292 collections. Of these 183 were locally confirmed as edible species and the edibility of 109 collections was confirmed from literature. Termite mounds accounted for the largest number, i.e. 157 collections and the remaining 135 were made from diverse habitats such as wood, coconut logs, roots of living plants, ground rich in plant litter/humus, etc.

Systematic taxonomic analysis of these collections revealed the presence of 87 species, 36 genera, 11 families

and 3 sub-orders. Nine agaric species, i.e. *Gyroporus* tax. sp (I), *Gyroporus* tax. sp (II), *Gyroporus* tax. sp (III), *Russula* tax. sp., *Boletus* sp., *Suillus* sp., *Tricholoma* sp., *Chlorolepiota* sp., and *Macrolepiota procera* showed ectomycorrhizal association with various plant hosts.

Studies on fruit body ontogeny in *Termitomyces orientalis*, *T. heimii f. rubescens f. nov.* and *T. striatus* showed a stipitangiocarpous type at hypogeal stage and bivelagiocarpic type at epigeal stage. The pigment in the pseudorrhizal rind of *T. orientalis* was found to be Melanin.

In all, 35 isolates were obtained from basidiome context tissue culture of 25 teleomorphs and a single isolate from the natural coremium of *Intromycopsis broussonetiae*, the anamorph of *Pleurotus cystidiosus*. Of the total 36 isolates, 18 belonged to 8 species of *Termitomyces*, 3 belonged to *Gyroporus*, 2 each to *Pleurotus*, *Volvariella*, *Hygrocybe* and *Panus*, and one each to the genera *Podabrella*, *Coprinus*, *Oudemansiella*, *Hygrophorus* and *Pluteus*.

Except for the powdery textured AVE-PMT and MOR-FN, all *Termitomyces* isolates generally showed good growth and uniform white, cottony morphology on PDA. AVE-5, R-23, COR-15, DHU-T-1, GUR-50, MOL-20 showed *in vitro* mycelial differentiation, giving rise to amorphous non-fertile clumps of plektenchymatic mass. Isolates from non-termitophilic species showed moderate to rapid growth.

Intercalary and terminal lemon-shaped mitospores and smooth-walled sphaerocysts were found in AVE-PMT and MOR-FN. Extracellular deposits of Calcium Oxalate were found in AVE-PMT on 0.1 percent TAMM. Asexual fructifications such as athroconidia or chlamydospores were detected in staling cultures of SC-209/88, GUR-50, VOLV-1/89, VOLV-1-4/88, SALV-1/89, BOL-1/89 and TU-1/90. Arthroconidia was found in PCYS-1. The black viscous exudate in VIC-1 was found to be Melanin.

All the isolates, except the two powdery ones, produced white mats in stationary liquid culture. Pelletisation was found to occur in shaken cultures of *Termitomyces* spp., i.e. AVE-5, HON-1, SUR-1, COR-15 and AVE-PMT after 7 to 21 days.

The cultures grew well at pH 5.5 to 6. Polyphenoloxidase and Cellulase activity was detected in the majority of cultures. The *Termitomyces* isolates were found to be slow colonisers of paddy-straw.

In vitro fructification in *Termitomyces albuminosa* isolate AVE-5 was positively obtained on two occasions on Fungal Comb Agar. These fruitbodies, Fb-I and Fb-II micromorphologically appeared similar to *Termitomyces* sp. Subsequently similar fruitbodies were consistently obtained on PDA under ambient conditions from the tissue cultured progeny isolate Fb-II-a.

(We greatly appreciate information from the participants in MUSHNET, particularly details of current activities and general information on the institutes and laboratories carrying out research on mushrooms. This information would be of prime interest to the MUSHNET participants and the readers of the *Genetic Engineering and Biotechnology Monitor*. We look forward to receiving further contributions in future. *Ed.*)