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Genetic Engineering and Bio-technology Monitor

Distributed free to a targeted audience in developing countries

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INTERNATIONAL GEN-TECH CENTRE ENTERS NEW ERA

Plenipotentiary Meeting Points The Way to Independence

Vienna, 8 December 1992

The International Centre for Genetic Engineering and Biotechnology (ICGEB) will take a major step towards independence when a plenipotentiary meeting on its future was held here today. With 26 countries having ratified the Centre's Statutes-the last of them being the Russian Federation-the aim is to bring these into force this coming year.

Operational since mid-1987 as a project under the auspices of the United Nations Industrial Development Organization (UNIDO), which fostered its creation and initial work since 1981, ICGEB became the first international centre to open up the enormous benefits of biotechnology specifically to developing countries in areas such as health, agriculture and energy.

During the plenipotentiary meeting, 16 countries signed the notification of the entry into force of the Statutes. It was agreed that the Statutes will enter into force on receipt by the United Nations Secretary General, as Depositary, of notifications from 24 States. Governments that were unable to accredit their plenipotentiaries to the meeting were invited to notify the Depository directly of their agreement to the entry into force of the Statutes.

When they enter into force, the Centre will be overseen by a Board of Governors consisting of its Member States. At the first meeting, scheduled for mid-May 1993, crucial issues relating to the long-term operation of the Centre as an autonomous entity will be discussed.

During the transition period, UNIDO is expected to play a key role in the transfer of responsibilities for the Centre to ICGEB's management.

ICGEB has already contributed to the battle against two major afflictions in the Third World. It is collaborating with an Indian Pharmaceutical company to make its diagnostic kit for AIDS commercially available in India and eventually in other developing countries. The Centre has also developed a peptide with the potential for use as a vaccine against hepatitis B while an agreement has been entered into with another Indian company for the commercialization of its research results in specific areas.

Since ICGEB became operational in mid-1987, its two components-in New Delhi and Trieste-have provided training for more than 900 scientists from 35 developing countries. Some 90 papers based on the Centre's research activities have been published in major scientific journals. It has also become increasingly involved in harmonizing approaches to biotechnology safety, intellectual property rights and patenting policies among its Member States. ICGEB is collaborating with affiliated centres in 17 countries in research and training.

Autonomy for the Centre will come at a particularly auspicious time since ICGEB is in the midst of a rolling five-year programme that has enabled it to recruit a core of highly-qualified scientists while imparting stability to its operations. A budget of $72 million has been committed for 1992-96.

The plenipotentiary meeting was opened by UNIDO Deputy Director-General Louis Alexandrenne, on behalf of Director-General Domingo L. Siazon, Jr. The President was Adolfo Taylhardat (Venezuela), who has served as Chairman of ICGEB's Preparatory Committee.

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A. NEWS AND EVENTS

UNIDO News

ICGEB to go independent

ICGEB is expected to become an autonomous organization as of 1 January 1993, according to the Centre's Preparatory Committee decision at the close of its seventeenth session held in Vienna from 19 to 20 February 1992.

Since its establishment in 1983, ICGEB has operated under the auspices of UNIDO, which fostered its creation and initial work. When its Statutes enter into force next year, the Centre will be overseen by a Board of Governors consisting of its member countries.

The next session of the Committee is scheduled for 5-9 October 1992, when it will confirm and finalize arrangements for a Plenipotentiary Conference to agree on the entry into force of the ICGEB Statutes.

The Committee approved the Centre's US$72 million work programme for 1992-1996, funding for which has already been identified.

Stemming from the recommendations of a working group on biotechnology safety made up of UNIDO, the United Nations Environment Programme, the World Health Organization and the Food and Agriculture Organization of the United Nations, the Committee called on the member countries to apply the Voluntary Code of Conduct on the Release of Organisms into the Environment formulated by UNIDO.

In this connection, the Committee stressed the potential contribution of UNIDO and ICGEB to this year's UN Conference on Environment and Development (UNCED) in the areas of biotechnology and harmonization of biosafety guidelines. (Source: *Helix*, May 1992)

UNIDO Draft Code on the Release of GMOs

(The previous issue of the Monitor (No. 39) contained the full text of the Draft Code. Hereunder is a comment from *African Diversity*.)

Since 1985 the Informal Working Group on Biosafety (a joint effort of the United Nations Industrial Development Organization (UNIDO), the United Nations Environment Programme (UNEP), the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO)) has been addressing issues of biosafety in relation to research institutions, industry and the environment. At its fourth meeting in 1989 the development and promotion of an *International Code of Conduct on the Release of Genetically Modified Organisms (GMOs)* was recommended. UNIDO was authorized to take the lead.

In 1991 UNIDO convened two meetings of some 50 scientists from the South and the North. The experts arrived at a draft Code of Conduct and the recommendation to establish an International Biosafety Information Network and Advisory Service within the United Nations system. Primarily the Service would provide advice and assistance in assessing the safety for health and environment of proposed GMO releases and applications. With the help of multidisciplinary expertise and links to national authorities and advisory boards the Service would compile information on assessed projects and assessment procedures world wide and make this guiding information available at national, regional and international levels.

The draft code puts emphasis on risk assessment prior to the release of GMOs and demands disclosure to the public. It identifies broad guidelines for release applications. Structures and procedures for application processing and the implementation of risk assessment strategies as well as follow-up are suggested.

However, a fundamental flaw of the draft is the assumption that the impact of GMOs can indeed be sufficiently pre-assessed. Considering the history of damage done by foreign organisms (not even genetically modified) introduced into new environments all over the world, considering the little experience there is in assessing the impact of newly introduced organisms in any environment, considering the limited knowledge there is on compositions of, and interactions within, the various environments and ecosystems in this world, this assumption seems questionable. The real problem of the release of GMOs - the risks and long-term effects we cannot imagine at the present stage of knowledge - does not figure in the draft code at all. If the code is implemented in its present version it may help pave the way for risky release of GMOs rather than prevent it. Given our limited current knowledge a moratorium on GMO release seems the need of the moment. (Source: *African Diversity*, No. 6, October 1991)

UNIDO's biotechnology initiatives in Africa

Unprecedented developments in the field of genetic engineering and biotechnology during the last few years have generated an immense interest in the industrial usage of living organisms. The term "biotechnology" was coined to encompass applications of this technology in such diverse fields as agriculture, improvements in animal and human health, and in energy. Since 1981, UNIDO has been actively involved in the major issue as to how the new technologies could be used for the benefit of developing countries in order to help solve some of their pressing problems and to advance their research capabilities and industrialization process.

In Africa, biotechnological research is in progress in several countries. However, levels of biotechnological capabilities vary widely within the continent. Variations in available local bioresources, scientific and technical infrastructure, low levels of international market development, and failures in setting of specific national priorities in science and technology have all adversely affected the development of African biotechnology. Lack of coordination and duplication of efforts is a problem. African countries often suffer serious technical and scientific isolation. There is an urgent need for more trained scientists and for the identification of specific projects that use available resources to solve problems relating to food, health and energy.

As developing countries grow aware of the potential benefits of biotechnology, some of them are beginning to take steps that will allow researchers to perform goal-directed research and development and encourage industrialists to capitalize on the results of both indigenous and foreign research. However, these efforts face severe constraints. The educational systems in Africa are not geared to the biotechnological education and training of the high quality, multidisciplinary researchers required. Many countries do not have adequate technological resources or the scientific competence to take up bioscience research and development or the technical capability to develop scale-up and downstream industrial processes. Capital investment funds for setting up a bioscience-based industry are usually lacking and indirect constraints often act to discourage industrial initiatives.

Many of these constraints can be overcome or circumvented through international cooperative actions. However, lack of resources prevents international organizations from undertaking large, wide-ranging programmes to bring biotechnology and its benefits to the developing countries. Nevertheless, past experience indicates that UNIDO can fulfil an important role by promoting projects that can have significant effects at local and regional levels. In particular, it is clear that benefits can accrue from the taking of steps to increase the capabilities of existing institutions performing R&D biotechnology. Accordingly, preliminary steps have been taken by UNIDO to establish and make operational a regional biotechnology network to serve the African continent. It would link existing R&D centres with possibly a few newly established centres in order to accomplish these major functions: they will perform in-house advanced biotechnology R&D of pertinence to the region; they will train researchers from African countries in advanced techniques of biotechnology; and they will act as focal points for the collection of scientific information and the further dissemination of the same to associated network R&D centres. An additional significant benefit of the network would be that research undertaken in the participating institutions can be coordinated, thereby avoiding needless duplication and other wastes of effort. Ultimately the value of the network to its participants will depend upon the content of its programme and the efficiency of implementation of its activities.

In the Lagos Plan of Action, the emphasis is on using science and technology as tools for sustained economic development. Highest priority in the process of development is for African nations to achieve self-sufficiency in food production; the second highest priority is to build up the industrial sector. The Lagos Plan of Action led to the Industrial Development Decade for Africa programme wherein a common framework was provided for guiding African countries in formulating development programmes and priorities within the context of individual countries' development plans.

The implication of biotechnology on African development was considered during the African Expert Group Meeting to Assess the Implications of New Technologies for the Lagos Plan of Action, held in Swaziland during 22-26 October 1984. One of the recommendations of the meeting was that African countries, at the national and regional levels, make a determined effort to "acquire, adopt and utilize the new technologies of recombinant DNA and of animal and plant tissue cultures" (see OAU document EDCO/ST/1/116/85). Furthermore, it was recommended that UNIDO explore the likelihood of setting up a biotechnology network to serve Africa.

The ICGEB

A major UNIDO initiative is the International Centre for Genetic Engineering and Biotechnology (ICGEB). The ICGEB is an international centre pursuing fundamental and applied research in molecular and cell biology for the benefit of developing countries. It is designed above all to serve as a resource for technology transfer through research training and expert advice to scientists in developing countries. In two research facilities in Trieste, Italy, and New Delhi, India, it houses a permanent staff conducting research in agrobiology and human health with special reference to problems of the developing world. The ICGEB provides an educational environment of international standard for visiting scientists from developing countries who spend a number of years at the Centre learning new techniques and participating in ongoing research. In addition, the Centre hosts and sponsors training courses, workshops and colloquia. It also serves as a source of expert advice and shares collaborative research with a wide network of affiliated research centres in its member countries. Eleven African countries - Algeria, Congo, Egypt, Mauritania, Mauritius, Morocco, Nigeria, Senegal, Sudan, Tunisia and Zaire - are members of the ICGEB, and three of these - Algeria, Egypt and Nigeria - have affiliated research centres. There thus exists the initial core around which the formation of an African regional biotechnology network can take place.

Country missions

In order to begin to formulate such a network, UNIDO fielded several expert missions in late 1989 to African nations that had requested aid from UNIDO in developing policies and programmes in the field of biotechnology. Consultants were sent to Senegal, Zaire, Kenya, Sudan and Ethiopia. Each mission was for a period of one to two weeks in the requesting country. The expert/consultant discussed with national authorities and scientists possible policies and programmes and prepared an outline of these, which included effective modalities for applying achievements made in genetic engineering and biotechnology. He advised on ways to apply biotechnology and genetic engineering techniques in economic, technological, scientific and social fields to help the country with the formulation of a national programme on biotechnology and on appropriate projects in biotechnology and training. Each consultant explored the possibility of the country participating in an African regional biotechnology network, identifying in particular the areas of cooperation that would benefit the country and advising on follow-up actions. Each expert then submitted a report to UNIDO, paying particular attention to scientific manpower resources, equipment, the extent of local support and finances and existing arrangements for collaboration, and the specific needs of the country.

Based on the foregoing reports and evaluations, UNIDO staff, with the help of short-term scientific/technical consultants, formulated a project for the creation of an African biotechnology network. The network is designed to link together scientific and technological capabilities, hitherto dispersed, which would find it easier to grow together, to keep in the forefront of international development in the field, and to introduce appropriate biotechnological innovations in their respective countries.

Biotechnology and food processing in Africa

In addition to this work, for several years UNIDO has actively been looking for ways in which African countries can benefit from advances in genetic engineering and biotechnology in applications appropriate to the food industry. Advances in genetic engineering and biotechnology offer a wide range of potential solutions to some of the basic food and nutrition problems facing developing countries. Genetic engineering can lead to increased food production through modification of the genetic make-up of plants, through the incorporation of nitrogen-fixing genes into cereal crops to make them less dependent upon chemical fertilizers, through increased resistance of plants to pests, and by increasing the nutritional value of foods through the insertion of desirable genes into specific crops. Fermented foods - foods in which micro-organisms are intentionally grown - are common in developing countries. Microbes are thousands of times simpler genetically than plants or animal cells, grow 70 to 80 times faster, and can be improved by genetic engineering or by simple scientific selection.

Genetic engineering will likely provide the tools required to accomplish needed changes in the processing of African foods. At least 25 per cent and perhaps as much as 60 per cent of the food produced in Africa is lost to insect, rodent and microbial spoilage. At least a portion of these losses can be avoided by proper harvesting and processing of the crops. Fermentation, widely practised in Africa, can lead the way to expanded food supplies in the form of Single Cell Protein (SCP) grown on inedible substrates, Microbial Biomass Protein (MBP) grown on edible substrates, and serve as a means of processing and preserving the food supply. Fermented foods such as Nigerian ogi (a weaning food for infants produced by the fermentation of maize, sorghum and millet), Kenyan uji, South African mahewu, Nigerian gari, cassava flour and fufu, which are obtained from fermented cassava, Ghanaian kenkey, and sorghum (Bantu) beer are important parts of the African diet. Other African food varieties obtained through fermentation processes include iru, produced by the fermentation of locust beans, cowpeas, soybeans or benniseed, and m'bannick, a drink obtained by fermentation of whole cow milk. Most of these fermentations can be upgraded, expanded and improved.

Given the acute nutritional problems that exist in Africa, and the potential for overcoming some of them through the application of biotechnological advances, UNIDO recently organized a regional Expert Group Meeting to consider specific areas in which modern biotechnology research and its results can be applied to food processing in Africa. The meeting took place at the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria, 16-20 December 1991, and built on ongoing activities in Africa in biotechnology and fermentation.

The overall objective of the meeting was to review constraints and offer potential biotechnology solutions to food production and processing in Africa. The meeting offered:

1. A review and consolidation of current research work in improving production of specific African food crops and food fermentation technologies. The review included opportunities for biotechnology in (a) pure culture fermentations, enzyme treatment, food detoxification; and (b) genetic improvements to food crops including cassava, oil palm, coffee, cocoa and plantain;

2. A discussion on constraints to the application of biotechnological advances to the processing of food in Africa and suggestions for solutions;

3. A consideration of ways to link African institutions and organizations to existing network arrangements in specific fields of biotechnology. Existing

networks include IITA and its linkages within the CGIAR system, to other advanced laboratories in Europe and North America, and to African research units, and the linkages between the ICGEB and its affiliated centres to African researchers and research institutions;

4. Suggestions of mechanisms for sustained follow-up on successful results of research and development in biotechnology and genetic engineering. Such mechanisms include the linkage of the ICGEB and its affiliated centres to work needed in Africa and to the training of African researchers in training courses and workshops. A linkage between advanced laboratories and African scientists will enable African countries to benefit from these new technologies of great potential.

The Expert Group Meeting was attended by 48 experts from 16 African countries, experts from other developing countries of Asia and Latin America, and experts from Europe, the United States, and IITA. The following projects for African countries which were suggested for follow-up are now being developed by UNIDO:

- A project on lactic acid fermentation technology for African scientists, to include training, pilot plant production and a network once they have completed training;

- The support of a Network on Food Fermentation and Processing Technology to stimulate research activities and information exchange within Africa and with other regions of the world;

- A project for transferring mushroom-growing technology on waste materials to Africa;

- A project to study and implement ways of improving indigenous cropping strategies of famine-stricken African countries by improving famine food continuance and stability;

- A project to disseminate technologies developed in Nigeria for soybean processing and utilization. These technologies, which have been widely accepted by Nigerian consumers, may be used to fortify African food, significantly improving its protein content;

- A project on developing a programme for Africans, primarily women, to develop skills in the processing of African foods that can be used by participants for improving nutrition in the home and be extended to the setting up of small-scale industries.

Country-level assistance

A project to provide preparatory assistance to Egypt to build a national programme in biotechnology and genetic engineering has been funded by UNDP and implemented by the Biotechnology and Genetic Engineering Unit of UNIDO. The purpose of the preparatory assistance was to formulate a project document for the establishment of a National Institute for Genetic Engineering and Biotechnology (NIGEB). Land has been acquired for building NIGEB and blueprints are being prepared for the Centre. The Centre is expected to be completed within two years and NIGEB has been nominated to be the ICGEB's affiliated centre.

In early 1992, UNIDO sent two consultants to Algeria to provide high-level technical assistance to help in formulating and implementing a national programme in biotechnology for the country, and to formulate a project for the establishment of a National Centre for Biotechnology and Genetic Engineering.

UNIDO's Programme on Bioremediation and Recovery of Oil

UNIDO has proposed the establishment of a research and development unit in oil recovery and bioremediation, a new technology that offers promise in rapid and environmentally safe clean-up of all manner of environmental industrial pollutants. The objective of the programme is to build national capabilities for developing countries, including the oil-producing countries in Africa, in these technologies. It will utilize training at one of the ICGEB Centres and the setting up or strengthening of two laboratories in oil-producing countries that specialize in petroleum research.

The Voluntary Code of Conduct for the Release of Organisms into the Environment

The Voluntary Code of Conduct for Biotechnology Safety prepared by the UNIDO Secretariat for the UNIDO/UNEP/WHO/FAO Working Group on Biotechnology Safety is being circulated, considered and discussed in all appropriate international and national forums. Support from many countries has been given to it and there are indications that, in some cases, it will serve as a basis for the formulation of regulatory guidelines for countries lacking regulatory policies in biosafety. In addition, UNIDO has been requested by many African scientists and research institutions in Africa to formulate and coordinate an advisory service network on biosafety issues to provide

expert advice, on request, to governments of developing countries lacking regulatory policies.

UNIDO studies plant-to-pharma opportunities

Industrial and governmental representatives from several countries met in Milan recently to try and resolve the practicalities of exploiting the planet's plant resources to make new pharmaceuticals and medicines.

The meeting, held under UNIDO auspices, took place just weeks before UN negotiators gathered in Nairobi to finalize a convention on the protection of biodiversity in time for the Earth Summit.

Italian firm Indena, Europe's largest producer of extracts and derivatives from plants, is said to have had a significant presence at the Milan workshop. Participants from Italy, Cameroon, China, Egypt, Guatemala, India, Indonesia, South Korea, Madagascar, Thailand and Turkey also took part in discussions, which were aimed at finding an approach to exploiting plant resources that would benefit all developing countries.

Specific issues included strategies for developing phytochemical and pharmaceutical industries, cooperation procedures for preserving and propagating medicinal and aromatic plant resources, the development of indigenous skills and technology for processing plants, and cooperation in the financing of R&D activities and joint venture projects.

Meanwhile, in Nairobi, around 300 lawyers and scientists began an eight-day session to finalize a convention to provide a legal basis for resolving such issues.

The fear is that without such an international agreement, the present rate of destruction of forests and other biodiverse regions will continue.

The trend not only exacerbates the problems of poverty, but, UNEP points out, means that many other cures for diseases may go undiscovered. Only one tenth of one per cent of all naturally occurring species have been exploited to date, it estimates.

At the very heart of the draft treaty is a highly controversial section concerning the management of financial resources. A UNEP statement says: "In the past, industrialized countries have enjoyed access to the biological resources of developing countries, but shared little or none of the profits with them." (Source: *European Chemical News*, 18 May 1992)

UN and other organizations' news

Biotech and UNCED

Political will and "considerable investment" will be required if biotechnology is to play a full role in sustainable development, according to a new report* compiled by the International Bio-industry Forum (IBF). The document, which was submitted to officials at the world environment summit this summer, was launched at an IBF meeting in Washington.

Over the next 50 years, the world's population is expected to double from its current level to around 10 billion. Biotechnology can help to meet growing demands for food, medicines, energy and raw materials, and to minimize the environmental impact of products, processes and waste, the IBF argues.

Biodiversity was one of the main topics for discussion at the UN Conference on Environment and Development in June, and the IBF also believes that biotechnology can help to increase genetic diversity.

However, these benefits might not be realized unless world leaders take coordinated action on four major points, the IBF contends. Regulations, policies and legal systems must be brought into line to protect investments. The industry also needs to be assured of adequate market access and reasonable returns. A "supportive" climate for technology - and for biotechnology in particular - must be nurtured. Finally, the industry requires effective protection for intellectual property rights.

The widening technological gap between North and South must be addressed through improved cooperation between governments, and between private industry and the developing nations, the report concludes. Regulatory structures in all countries should "convey trust and confidence for all parties involved". (Source: *Chemistry and Industry*, 6 April 1992)

WHO: fighting tropical diseases at low cost

About 50 programmes of the World Health Organization (WHO) have a significant research component, often including the search for new or improved biotechnology. Among them, the Special Programme for Research and Training in Tropical Diseases (TDR) is one of the largest. This TDR programme not only focuses on tropical diseases, but also recognizes that capacity building in developing countries can be essential for achieving health objectives effectively. More information may be had by referring to *Biotechnology and Development Monitor*, No. 9, December 1991, or the World Health Organization, 1211 Geneva 27, Switzerland. Tel. (+41 22) 7912111, Fax: (+41 22) 7910746.

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* "UNCED '92: Policies for sustainable development - the role of biotechnology", SAGB, Avenue E. Van Nieuwenhuyse 4, bte 1, B-1160 Brussels, Belgium.

GATT and the legal protection of plants in the third world

If the draft final Act, the provisional result of the Uruguay Round of negotiations under GATT, is unanimously to be accepted in its present form, developing countries are obliged to confer legal protection for plants, either by the patent system, or by some kind of plant variety protection system. It would be a curious outcome. No developing country has really asked for such an obligation. Hardly any evidence exists on the impact of intellectual property protection of plants on developing countries, and there is no guarantee that the pressure of the American Government on individual countries to recognize patents on plants will come to an end. For more details, refer to *Biotechnology and Development Monitor*, No. 10, March 1992.

Animal Genetic Resources Programme

The United Nations Food and Agriculture Organization (FAO) is preparing to launch a five-year, $15 million Global Animal Genetic Resources Programme that aims to rescue such unlikely animals as the Shiwal cow of Pakistan, the Taihu pig of China and the Fayoumi chicken of Egypt. Each is threatened not by predators or lost ecosystems, but by established Western breeds such as the Holstein cow and the factory chicken. Hoping to increase their output, farmers in developing countries are switching to high-productivity Western animals, abandoning native breeds that have been adapting to the local conditions for decades. FAO worries that if the local breeds are allowed to disappear, their disease-resistance traits and ability to withstand harsh conditions may be lost, too.

Although Western farm animals generally produce more milk, eggs or offspring than the hardened stock of Africa, Asia and Latin America, experience has shown that there is nothing like an epidemic or a heatwave to remind local farmers why the old breeds have been around for so long. The Chinese Taihu pig may not grow to the size of a typical Western sow, but it can live on cabbage if need be.

The tropics are likely to be the most critical area. Because that is where human populations are growing fastest, tropical farmers are under the greatest pressure to improve production. Switching to a high-output Holstein may help for the moment, but FAO is worried about the possibility of disease or drought; pathogens are being spawned as fast as people in that part of the world. Researchers say that it is only a matter of time before one of them emerges to wipe out the Western stock. By then, the disease-resistant traits of some once-local breed may have already been lost.

FAO proposes to use about a dozen species - including the Barbados Black-belly sheep, the N'Dama cattle of West Africa and the South American Criollo cattle - as trial projects in field preservation. Other endangered breeds would be added as they are identified.

DNA "fingerprinting" of rare breeds can indicate which are distinct enough from more common stock to merit preservation. And as geneticists continue to identify disease genes, it may soon be possible to find out which breeds have the most valuable resistance traits. Several groups - including those at the Indian Veterinary Research Institute, the University of Brisbane and Texas A & M University - are already investigating livestock genetic resources. FAO hopes to get more researchers to join them down on the farm. (Source: *Nature*, Vol. 355, 30 January 1992)

OECD expert group on safety in biotechnology

The OECD group of national experts (GNE) on safety in biotechnology met in Paris in December 1991, the plenary preceded by more specialized meetings in Leiden and Paris on field release trials (especially of rDNA plants). Some 120 experts were present, from almost all the OECD's 24 member countries. Papers and proceedings are restricted.

To be published shortly is a two-part report on "GILSP" (Good Industrial Large-Scale Practice) and "GDP" (Good Development Principles - for the conduct of small-scale field trials). The December 1991 and follow-up work is focusing on field trial scale-up, for plants, but also for micro-organisms and later on for animals.

Recommended for derestriction are the report on a December 1990 Copenhagen workshop on monitoring techniques for genetically modified organisms; and a rapidly and recently-completed report on the safety of foods produced by (terrestrial) biotechnology (group chaired by Dr. F. Young of the US Department of Health and Human Services). A workshop on the safety of foods from marine organisms took place in Bergen, Norway, in June 1992.

Also recommended for derestriction is the BIOTRACK database, in which staff of the Environment Directorate have collected in standard format details of some 500 "release events". More details will be reported in *EBIS* once publication is cleared.

The meetings of GNE and its sub-groups reveal differences, not only of scientific opinion, but of regulatory approach, reflecting ultimately differences in political and public perceptions. Such differences, however, are inevitable; and while making scientific consensus more difficult to attain, their existence increases, rather than diminishes, the need for and the value of the GNE work.

Details: GNE: Ms. Bruna Teso, Head of Biotechnology Unit, OECD, 2 rue André Pascal, F-75775 Paris. Tel. (33) 14529331; Fax (33) 14529767.

BIOTRACK: Dr. Vic Morgenroth, Environment Directorate, OECD. Tel. (33) 145249775; Fax. (33) 145241675.

(Source: *EBIS*, Vol. 2, No. 1, 1992)

Social issues

Bioethics: Europe drafts a convention

Europe has taken an important step towards making bioethics a legitimate topic of political debate, as well as a universal right that governments must respect.

Meeting in Madrid, the chairmen of the ethics committees from the 26 countries that make up the Council of Europe adopted a draft of a European Convention on Bioethics. Participants also agreed to become members of a standing conference that would promote discussion and raise awareness of their work.

The convention - due to be ready by the end of 1993 - will consist of a framework of fundamental principles, based loosely on the European Convention on Human Rights. It will incorporate respect for human dignity, protection of individual integrity and the prohibition of all commercial agreements concerning the human body and its organs. Subsequent protocols will contain the rules for specific fields of bioethics, the first two of which will cover organ transplantation and biomedical experiments on humans.

The terms of both the convention and the protocols are expected to be quite general. While such broad wording will not be very useful as a guide to decisions on specific cases, observers believe that the documents will serve to promote the discussion of bioethical issues across Europe. In addition, the convention is unlikely to eclipse national legislation in deciding what researchers are permitted to do.

The Standing Conference of National Ethics Committees will provide much-needed channels of communication and allow bioethical issues to be debated on a pan-European scale. Catherine Lalumiere, Secretary-General of the Council of Europe and proposer of the conference, says that, in particular, bioethics must be discussed in the context of economic as well as scientific and legal issues.

Meanwhile, in Paris, three draft bioethics bills were presented to the French Council of Ministers for their consideration before being passed on to the spring session of Parliament. Few changes are expected to be made to the bills, which are couched in terms similar to the draft European convention. The French Government hopes that this body of legislation, once passed, will become a reference point as its European neighbours and the United Nations discuss medical and genetic human rights. In 1983, France was the first country in Europe to set up a national ethics committee.

In London, the Nuffield Council on Bioethics announced that a panel has been formed to examine genetic screening. The group will look at the techniques involved, their benefits and difficulties, and such ethical issues as the handling and holding of information and consent to being screened. The panel will report to the council - which has no legislative power - within 18 months. (Source: *Nature*, Vol. 356, 2 April 1992)

EC bioethics group

The Commission of the European Communities has set up a Group of Advisers on the ethical implications of biotechnology. The members of the Commission, which met first on 9-10 March, are as follows:

- Mme Noelle Lenoir (Membre du Conseil Constitutionel, France)
- Dr. Margareta Mikkelsen (a doctor and President of the European Society of Human Genetics, Denmark)
- Professor Marcello Siniscalco (Professor of Genetics and Adviser to the National Council of Research, Italy)
- Lady Warnock (philosopher, University of Cambridge, UK)
- Mr. Marcelino Oreja (magistrate and President of the Institutional Committee of the European Parliament, Spain)
- Professor Hans Zacher (President, Max-Planck Institute, Germany)

(Source: *Biotechnology Bulletin*, April 1992)

Gene therapy cleared by Government Committee

The treatment of fatal, inherited diseases by gene therapy poses no new ethical problems, according to a new report by the UK Committee on the Ethics of Gene Therapy. Gene therapy is desirable, the Committee concludes in its report to the Department of Health, and is in principle no different from organ transplantation. Committee chairman Sir Cecil Clothier stressed: "As a lawyer, I would deprecate any sort of legal control [on gene therapy] at this stage, because we don't really know what we are controlling."

Patients most likely to benefit from gene therapy are sufferers from genetic disorders where a single gene is thought to be responsible - for example, cystic fibrosis or Huntingdon's disease. The aim would be to repair or replace the faulty gene in living, non-reproductive cells, so that its function is restored. Attempts to modify germ cells - sperm or ova - are ruled out for the moment, although the idea of germline therapy is not dismissed

for the long term. The report was published by HMSO in January 1992, price £6.90. (Source: *Biotechnology Bulletin*, February 1992)

Nuffield Council calls for observations

The UK's Nuffield Council on Bioethics (London) has called for parties interested in genetic screening to submit their observations to the council's working party on the subject. It seeks information on advances in genetic screening and its applications and on benefits and difficulties experienced or foreseen, as well as on the ethical issues surrounding genetic information storage, confidentiality, stigmatization of the genetically disadvantaged, and employment-related and insurance-related issues. (Source: *Bio/Technology*, Vol. 10, May 1992)

General

French find short cut to map of human genome

International efforts to decode the human genome will be speeded up dramatically by a powerful new technique for handling long stretches of DNA. The researchers in Paris who invented the technique say they will have "mapped" about 90 per cent of the human genetic blueprint before the end of this year.

Daniel Cohen and his colleagues at CEPH, the French centre for genetic research, have pieced together a map covering 25 per cent of the genome, including virtually the whole of chromosome 21. Cohen is confident that his map will accelerate the hunt for genes linked to Down's syndrome, Alzheimer's disease and certain forms of epilepsy.

Building physical maps of chromosomes out of cloned stretches of human DNA is a key first goal of the international genome programme. The complete genome is simply too vast for geneticists to search through every time they wish to trace an interesting gene or fragment of DNA.

Mapping solves the problem by carving the genome into a jigsaw of manageable fragments. Each fragment is analogous to a book in a library whose shelf mark has been clearly identified. The problem of finding a gene is then reduced to that of finding a quote in a single book rather than having to search through a whole library.

Elsewhere, geneticists are close to completing maps of the X and Y chromosomes that encode sexual characteristics. Cohen predicts that it will be three or four years before the human genome is mapped completely. The last 10 per cent of the DNA will be the hardest to clone, he says, because it will be widely scattered as a series of small "gaps" in the map.

In contrast to the huge National Genome Project in the US, which is funded by the Government, 70 per cent of the money for Cohen's research comes from a French muscular dystrophy charity, AFM, which has set up a laboratory in Paris dedicated to mapping the human genome.

The technology propelling the French effort is the "megaYAC", an artificial chromosome that can store up to one million base pairs of DNA. The French map of chromosome 21 is spread over 250 megaYACs. A complete map of all three billion base pairs of the human genome would require more than 30,000 megaYACs, says Cohen.

The megaYAC's forebear, the yeast artificial chromosome, was invented in the late 1980s for cloning stretches of DNA that are too long to be manipulated in bacteria. A conventional YAC can only hold up to 200,000 base pairs of DNA: it is the fivefold expansion of this capacity which makes the megaYAC such a boon to genome mappers.

Researchers in the United States and Britain have provoked criticism in the past six months by patenting stretches of DNA of unknown function. Cohen has decided not to patent the DNA in his map, which will be opened to all researchers in July. (Source: *New Scientist*, 23 May 1992)

European collection of animal cell cultures (ECACC)

ECACC was established in 1984 and is recognized as an International Depository Authority (IDA) under the Budapest Treaty. It accepts animal cell lines and viruses from all over the world. Cell lines or cultures can be received pre-frozen or growing, to be frozen in its own laboratories, under the strictest confidentiality. The cell lines available for world-wide distribution include those stored by the ECACC and the European Human Cell Bank.

The collection's experience of handling cell lines enables it to offer a wide range of cell characterization, standardization and validation. All of these services can be carried out to Good Laboratory Practice (GLP) standards. Details from: The European Collection of Animal Cell Cultures, Division of Biologics, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury SP4 0JG; or on 0980 610391. Fax: 0980 611315.

ATCC catalogue of human and mouse DNA probes and libraries

In its fifth edition catalogue, the American Type Culture Collection (ATCC) describes over 2,600 human and mouse DNA probes and libraries available from the

ATCC's Repository of Human and Mouse DNA Probes and Libraries. The Repository is supported by a contract by the National Institutes of Health (NIH).

Details from: Patrick J. Burke, ATCC Marketing, ATCC, 12301 Parklawn Drive, Rockville, Maryland 20852-1776, USA or on +1(301) 881 2600. Fax: +1(301) 770 2587.

Mutant bacteria may escape from the mail

Potentially dangerous bacteria are regularly sent from one laboratory to another - through the post. The mailed micro-organisms include genetically modified bacteria, yet a survey in the Netherlands reveals that not one laboratory packed its samples properly. Damaged packages could leak genetically altered organisms into the environment, say the Dutch researchers.

The UN's Committee of Experts on the Transport of Dangerous Goods lays down rules for posting samples of "viable micro-organisms ... known or suspected of causing disease in animals or humans". The International Air Transport Association, and several national laws, including Holland's, extend these rules to any genetically modified micro-organism.

The rules state that samples must be enclosed in two watertight containers, with enough absorbent material between the two to soak up the contents of the inner container should it break. In Holland the outer package is supposed to be labelled "health risk due to genetically-modified material (biohazard)".

Scientists at the Dutch National Institute for Public Health and Environmental Protection requested samples of genetically modified micro-organisms from 10 laboratories in Holland, the United States, Australia and Singapore and treated them to the sort of treatment they might expect in transit.

Not one of the packages bore a "biohazard" statement; and none conformed to the UN or Dutch specifications. Four contained glass tubes, while one simply contained a plastic Petri dish wrapped in plastic film.

The Dutch microbiological culture collection in Utrecht receives 200 bacterial samples a year, 90 per cent of which are genetically modified. Half of them are sent in plastic Eppendorf tubes, which did not survive being trodden on. About one sample each year arrives in Utrecht in a broken container.

The Dutch scientists estimate that each year Dutch laboratories post 3,000 packages containing modified bacteria. Given the frequency of breakages reported by the culture collection and the national bacteriological laboratory, this means there could be 15 accidental introductions of modified organisms a year into the environment in Holland alone. The team concludes that packaging standards "are insufficient to protect against possible risks". (Source: *New Scientist*, 4 April 1992)

New *in vitro* diagnostics trade association formed

The British In Vitro Diagnostics Association (BIVDA) has been founded to meet the specific interests of the *in vitro* diagnostics industry, currently represented under the umbrella of the other larger trade associations with diverse interests. BIVDA aims to fulfil the need, long recognized by the industry, for representation at the European level by an organization whose sole focus is in the *in vitro* diagnostics industry. The association was formally set up at a meeting of a significant number of the UK's leading *in vitro* diagnostics manufacturers in February 1992. The founder members have appended their names to a letter being sent to all UK *in vitro* diagnostics companies inviting them to join and asking whether they will accept BIVDA representation on the European Diagnostics Manufacturers' Association. A secretariat has been organized within the offices of the BioIndustry Association at 1 Queen Anne's Gate, London SW1H 9BT, Tel.: 071 222 2809, Fax: 071 222 8876. (Source: *News Release*, 14 February 1992)

WARDA: rice research in West Africa

The West African Rice Development Association (WARDA) is currently implementing a new research programme to address the emerging gap between rice demand and production in West Africa. Biotechnology applications may become important in the longer term, as the African rice germplasm collection is transferred to WARDA. The share of rice in West Africa's consumption of food grains is rising at a rate of 4 per cent a year, faster than that of any other food crop except wheat, as consumers switch away from the region's traditional staples, maize, millet and sorghum. The increasing demand for rice is largely met through imports, facilitated by the low world market prices for rice. Cheap imports widen the gap between domestic supply and demand still further. They discourage domestic rice production and fuel demand by broadening it across social classes: lower-income groups are becoming new consumers of rice. While the annual growth rate of regional rice production exceeded 3 per cent in recent years, this rate has been largely due to an expansion of cultivated area. Regional rice yields, which average only 40 per cent of the world mean, have been stagnant.

To address the emerging gap between regional rice demand and production, WARDA's initial approach was based on field trials presuming that significant gains could be achieved through the direct introduction of materials developed at the International Rice Research Institute (IRRI). However, it was soon clear that Asia's Green Revolution could not be easily transferred to Africa. Irrigation is too limited in extent, and smallholder farmers in West Africa rarely have access to

the inputs used elsewhere in the world. Fertilizer use is particularly low. As a result, WARDA decided in 1983 to initiate its own breeding programmes in all ecosystems it addresses (breeding had already begun in the Mangrove Swamp Programme as early as 1976).

WARDA was formed in 1970 with the assistance of the United Nations Development Programme (UNDP), the Food and Agriculture Organization of the United Nations (FAO) and the Economic Commission for Africa (ECA). WARDA's mandate is to assist its member countries to become self-sufficient in rice, a staple food of West Africa. It is an intergovernmental organization presently consisting of 16 countries: Benin, Burkina Faso, Chad, Côte d'Ivoire, The Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone and Togo.

The Association became a member of the Consultative Group on International Agricultural Research (CGIAR) in 1986.

Research at WARDA has been organized into three priority programmes, related to the three major rice-growing environments in West Africa:

1. The Upland/Inland Swamp Continuum Rice Research Programme, located at Côte d'Ivoire headquarters;

2. The Sahel Irrigated Rice Research Programme, located in Senegal;

3. The Mangrove Swamp Rice Research Programme, located in Sierra Leone.

In each environment emphasis has been laid on developing cultivars with improved tolerance or resistance to the major biotic and abiotic stresses, including insects and diseases, cold, and adverse soils (salinity, acidity, and iron-, aluminium- and manganese-toxicities).

It is expected that through the formation of networks, some of the problems will be solved that currently hamper the transfer of new technologies. Feedback from on-farm trials and national programmes in recent years indicated that WARDA has succeeded in selecting and developing a number of higher yielding and more stable rice varieties that are well adapted to mangrove swamp conditions. Three varieties show tolerance to a wide range of abiotic and biótic mangrove swamp stresses, such as salinity, iron toxicity, blast and stem-borers. However, the transfer of these varieties to national programmes, their subsequent multiplication and their adoption have remained limited. (Source: *Biotechnology and Development Monitor*, No. 10, March 1992)

World AIDS programme "lacks vision"

The Global Programme on AIDS (GPA) at WHO should be radically overhauled, says a report to be published later this year. An independent review of the programme will recommend that other agencies, such as the United Nations Development Programme (UNDP) and the World Bank, must take greater responsibility for AIDS.

The report will recommend that in the long term WHO should withdraw much of its direct support to AIDS programmes in member States so that it can concentrate on advising their health ministries and coordinating other bodies.

A summary of the report, which has been seen as a strong criticism of the GPA, has been circulated to interested parties in advance of the full report.

The review was commissioned in 1989 by the GPA's management committee, whose members include the WHO's donor countries, other agencies, and delegates from the WHO's regional offices. (Extracted from *New Scientist*, 1 February 1992)

Third world countries should control their genetic resources

Developing nations should declare sovereignty over their own genetic resources. Such action will help protect the planet's dwindling biodiversity and allow poor countries to profit from growing commercial demand for plants and animals.

This recommendation comes from the Global Biodiversity Strategy released in Caracas, Venezuela. The blueprint for conservation, prepared by the World Conservation Union (IUCN), the United Nations Environment Programme and the World Resources Institute, is meant to complement the International Convention on Biodiversity. The strategy should be a basis for practical action until the convention is brought into force, say the authors.

The strategy argues that advances in biotechnology and increased interest of plant breeders and drugs companies in genes from wild species call for a reassessment of the notion that genetic resources should be treated as a common heritage, open to all.

The document urges countries to assert control over the exploitation of their genetic resources through national laws and regulations. However, it is not necessary for nations to immediately solve the complex question of "physical ownership" of wild plants and animals, it says.

Royalties, fees and taxes should be levied on the use of genetic resources, says the strategy. This would allow poor countries to capture some value from the resources while providing an incentive for conservation. For example, plant breeders and pharmaceuticals companies should be required to negotiate contracts with local people whose knowledge helps them to collect useful species. Fees should, likewise, be paid by international companies to local institutes such as gene banks which collect, identify or screen wild plants. And taxes should be paid by commercial enterprises that profit from the genes, the strategy recommends. (Source: *New Scientist*, 15 February 1992)

The green gene machine

Like conventional plant breeding, agrichemical R&D, and economical and environmentally sensitive farm management practices, plant biotechnology will play a major role in assuring a plentiful and safe food supply and in meeting world food production needs.

Plant transformation

The first transgenic plant was produced nearly 10 years ago. Today, nearly 50 species of crop plants can be genetically manipulated. The list includes nearly all major dicotyledonous crops and a rapidly increasing number of monocotyledonous crops, including rice and corn. Current research will probably lead to routine gene transfer systems for nearly all major crops within two to three years. Technical improvements will further increase transformation efficiency, extend transformation to elite commercial germplasm and lower transgenic plant production costs.

Gene expression

Plant genetic engineers currently have in hand a large battery of regulatory sequences that provide for both constitutive and highly tissue-specific gene expression within transgenic plants. Moreover, established differential screening methods permit ready isolation of regulatory sequences (e.g. seasonal, climatic or stress-related) for even more sophisticated expression control. Genetic engineers can also turn off endogenous genes. Striking phenotypic alterations follow selective inactivation of genes by the transcription of antisense genes. Researchers are seeking still higher levels of organ-specific gene expression in order to make specialty chemical or pharmaceutical production in plants more economically viable. They also want to insert genes in a more site-specific manner to minimize the variability of gene expression among transformants.

Gene discovery

Advances in identifying and isolating new gene coding sequences are important in engineering improved plants. The interspecies-specific use of transposons and T-DNA insertion permits tagging and isolation of novel genes from several plant sources. The availability of high resolution physical maps in tomato and *Arabidopsis* has already led to mapping of several novel loci, and new methods will allow direct testing of the isolated DNA for its ability to complement the mutation of interest at each step during chromosome walking. Advances in the redesign of coding sequences for plant expression allow for predictable, high-level expression of a variety of non-plant genes in crop plants.

Gene stability/germplasm access

The overwhelming conclusions from nearly 500 field test experiments on genetically engineered plants in the United States and Europe are that newly introduced genes - including those for quality improvement and for control of insects, weeds and plant diseases - are stable, inherited and are expressed like any other plant gene. Such traits have already been successfully introduced into several important crop species; genetically engineered soybean, cotton, rice, oilseed rape, sugar beet, tomato, alfalfa, potato and corn crops are expected to enter the market between 1995 and the year 2000. Broad germplasm access is likely to require extensive backcrossing or micropropagation methods.

Thus plant biology is set to enter a period when both basic research and commercial applications are limited only by research creativity and by funding. (Source: *Bio/Technology*, Vol. 10, January 1992)

Boom time for biotechnology

Biotechnology was the fastest growing area of scientific activity of the 1980s, if the number of papers published in the field is a reliable indication of growth. In a league table compiled by the Philadelphia-based Institute for Scientific Information, the number of papers in biotechnology and applied microbiology proliferated from 511 in 1981 to 2,373 in 1990 - an increase of 364 per cent. Trailing a poor second, with an increase of 129 per cent, is anaesthesia and intensive care.

The ISI published the table in *Science Watch*, a bulletin that monitors trends and performances in basic science. The institute scanned 3,700 journals covering all fields of science. The average increase across all fields was 53 per cent. Mathematics showed the least movement, with an increase of just 22 per cent.

Various fields of medical science occupied fifth to tenth places in the league, reflecting the huge increase in medical and biomedical science over the decade. In the United States, for example, one third of all scientists are now engaged in the life sciences.

Molecular biology and genetics occupied fifth place in the league table. All the other medical fields in the top ten were relatively specialized areas of clinical

research, such as urology in ninth place and orthopaedics and traumatology in eighth.

The only two non-biological sciences in the top ten were communications and data processing in third place and materials science in fourth. The number of published papers in these two fields increased by 115 and 113 per cent respectively. Fields such as general physics (+35 per cent) and general chemistry (+25 per cent) grew slowly because they are "mature" sciences that have spawned many new and exotic fields of research. (Source: *New Scientist*, 11 January 1992)

First International Symposium on the Biology of Adventitious Root Formation, Dallas, Texas, 18-22 April 1993

The First International Symposium on the Biology of Adventitious Root Formation will be held at the Richardson Hilton and Towers near Dallas and will be comprised of several sessions dealing with various aspects of root formation in conventional cuttings, tissue cultures, or whole plants. The following topics will be covered by invited speakers: biological diversity of adventitious root formation (P. W. Barlow, UK), commercial importance of adventitious rooting (J. L. Kovar, G. S. Foster, F. T. Davies, USA), applicable developing technologies for rooting research (S. G. Ernst, USA), assessment of plant materials for rooting research (D. E. Riemenschneider), novel experimental systems for determining cellular competence (D. Mohnen, USA), anatomical and cytological markers (W. P. Hackett), biochemical and molecular markers (P. Hand, UK), manipulating rooting potential (B. H. Howard, UK), growth regulators and the environment (A. S. Andersen, Denmark), auxin transport and metabolism (D. Blakesley, UK), auxin receptors and binding (K. Palme, Germany), genetic transformation with rol genes (D. Tepfer, France), root specific genes (J. D. Hamill, Australia), modelling root system development in herbaceous plants (S. Morita, Japan), modelling root system development in woody plants (D. I. Dickman, USA), expert systems for modelling propagule development (H. M. Rauscher, USA), carbon allocation to roots and shoot systems (A. Friend, USA), biology of hardening (R. Harrison-Murray, UK). Several panel discussions will also be held. Each invited presentation will be published as a full-length paper in the proceedings of the symposium. Contributed papers will be presented in poster sessions and published as abstracts. Due date for abstracts is 1 November 1992. Registration is due 1 February 1993. For more information on the symposium contact: Edith Franson, Executive Secretary, Rooting Symposium, USDA Forestry Sciences Laboratory, Box 898, Rhinelander, WI 54501, USA. (Tel.: 715-362-1112; fax: 715-362-7816). Chair, Executive Committee: Bruce E. Haissig; Chair, Organizing Committee: Tim D. Davis.

Biopesticides aim at a healthy market

Biopesticides are finally beginning to realize their commercial and technical potential. With a larger number of better products now being sold at prices more competitive with chemicals, the market is growing. Factory gate sales presently range from $45-60 million, an increase of around 80 per cent in the past three years.

Although the agrochemical market has grown very slowly to about $23 billion, biopesticides sales, still a tiny percentage of the total, have grown much faster. Potential markets for biopesticides have been exploited or identified in most agricultural sectors, including food crops, plantation crops, fruit production, forestry and grasslands. More than 80 companies are presently involved in biopesticides.

Chemical companies often examine biopesticides' potential in an effort to make themselves more environmentally friendly and to prepare themselves in case a chemical-free future ever comes. Other entrants hope to use spare fermentation capacity, or to extend their interests into crop protection, animal husbandry or biological products.

Biotechnology companies enter with the belief that their approach will be more focused, or more flexible, or that they have greater technical expertise, or have the rights to a new super-strain. Other new companies hope that their new technology, clever production or formulation techniques will give them the edge that has eluded others. One new entrant began with the conviction that professional marketing expertise would transform the hitherto amateur marketing of biopesticides.

Interestingly, experience suggests that few of these reasons have yet made much difference to success or failure. The real keys to success are:

- Production capacity and skills - success so far has required in-house production and putting process and production development as top priorities in R&D;
- Market understanding - the successful companies have staff with experience of the unique problems of marketing biopesticides. Marketing strength in agrochemicals has not to date been a satisfactory substitute:

- Corporate commitment - success in biopesticides does not come without effort. A vacillating or half-hearted corporate approach has invariably led to disillusionment, disappointment and failure.

New biotechnology companies have all started selling products and many of them, including Mycogen, Ecogen, EcoScience and Bactec have raised money for further growth.

Technology is developing rapidly with new formulations and improved production generating more cost-effective products in many sectors including forestry and vector control. Genetic engineering and non-recombinant techniques have produced novel *Bacillus thuringiensis (Bt)* strains with increased insecticidal activity, as well as new formulations.

Conjugation and partial curing are being used to generate strains with novel combinations of activities, some of which are in new products. Although, historically, non-recombinant methods have raised fewer regulatory issues than the use of genetic engineering, the distinction is now becoming less important.

Recombinant technology has been used to insert a *Bt* toxin gene into different microbial hosts including *Escherichia coli, Bacillus subtilis, Pseudomonas*, an endophytic bacterium and blue-green algae. The first genetically engineered products were registered in mid-1991 and are now on sale.

Worries over possible insect resistance to *Bt* are real but are being responsibly addressed by both industry and government and do not appear to spoil *Bt*'s new dawn.

However, products based on many other micro-organisms and nematodes are challenging *Bt*'s position as the world's sole commercially significant biopesticide. Although many attempts over a long period have failed to make commercial products using *Metarhizium anisopliae* and *Beauveria bassiana*, new patented production methods and new formulations will finally launch these fungi on to western markets.

The registration and protection of intellectual property rights (IPR) for both natural and genetically manipulated biopesticides are becoming easier with the ongoing harmonization in the European Community. The tier system of testing has become generally accepted internationally: both naturally occurring and genetically manipulated organisms have been approved for field testing and, more recently, commercial sale.

Meanwhile, patents, trade marks and trade secrets are all being effectively used to protect IPR and create entry barriers, making the potential markets more attractive to the companies that succeed. (Source: *European Chemical News*, 22 June 1992)

Sustaining biodiversity: Taxing biotechnology?

One of the key concerns of the United Nations Conference on Environment and Development (UNCED) was the negotiation of a biodiversity convention which would help to slow down the alarming rate of loss of plant and animal species on the planet.

The sudden and dramatic loss of biodiversity in recent decades is largely caused by habitat destruction, over-harvesting and pollution. The threat of global warming could further accelerate the loss.

What the biodiversity convention sought to accomplish was to set out rules for sharing information on our global biodiversity and establish effective countermeasures to protect it. It also called for improved plant, animal and fish breeding through traditional and modern biotechnologies to improve yields for consumption.

Clearly, the preservation of species' natural habitats is the surest way to ensure the planet's sustained biodiversity. But there are other solutions that are also necessary and effective. Getting farmers and governments to participate in the preservation of plant species in seed banks is one solution. But even this gets very complex and has raised issues that lay at the heart of some of the debates in the UNCED negotiations.

Some 20 per cent of the world's valuable plant species are already preserved in gene banks, such as those of the Consultative Group on International Agricultural Research (CGIAR) and its network of 16 international research centres, which hold the seeds in trust for developing countries.

These seed banks have raised the difficult issue of who should profit from the use of these seeds, especially in their manipulation through biotechnology. During the Green Revolution, for example, the banks used their existing seeds to breed new high-yielding varieties that maximized the use of fertilizers and pesticides and were seen to benefit Northern chemical companies.

Activists say that while many of these plants originally came from developing countries (like rice from India and China and cotton from Egypt), it is mainly the seed companies that own the new varieties, together with the fertilizer and pesticide companies, that profit from their manipulation.

Meanwhile, the activists say, the farmers in developing countries - the key players who bred and preserved many of the plants that we use today, from apples to zucchini - receive no compensation for centuries of keeping the food and seed stock healthy. It is this effort, often part of knowledge, innovations and practices of indigenous and local communities and embodiment of traditional lifestyles, that must be sustained if biodiversity is to be maintained.

Critics argue that if companies are to be paid for their work, so should the people who developed the plant species that the companies are now working on to improve further.

In the biodiversity convention discussions as well as in several other areas of discussions within UNCED, countries are fighting over whether there should be some form of payment for these farmers to continue their work, and whether there should be a link between maintaining biodiversity and the engineering of new strains using biotechnology.

Developing countries say that there is a clear link and that if developed countries want them to pay for new varieties that they have bred they should pay for the original species that came from their countries or at least pay to preserve the healthiness of this stock.

Developing countries are in danger now of having to pay out even more without any compensation as biotechnologists in the West are breeding yet more productive plants that will be crucial for raising crop productivity.

One innovative plan to pay for the efforts of farmers and agronomists in the South to protect biodiversity is a fund that the 150 members of FAO agreed to set up last November. It could be implemented as soon as the international convention on the conservation of biodiversity is successfully negotiated.

In principle, the FAO international fund will collect money from the users of the plants, such as the seed companies, to pay into a global system. This fund would in turn be used to finance local efforts to preserve plant species for the use of humanity, according to José Esquinas-Alcazar, the secretary of FAO's Commission on Plant Genetic Resources.

For example, several Northern Governments are very keen to promote private biotechnology industries and have recently relaxed rules on developing new strains to spur growth in this field.

At the same time they jealously guard the "intellectual property rights" of companies that develop new strains and their rights to profits from their research and development as well as those of researchers who go into the jungles to discover new plants with medicinal or food value.

But the issue is not one of ownership. This concept is fundamental. The species must be protected and used for humanity, not simply for commercial gain, although research and preservation work must be paid for.

The FAO proposal for a global fund would pay for the preservation and return of this healthy stock to all farmers, regardless of the wealth - or poverty - of the biodiversity of their particular region. (Extracted from *Development Forum*, May-June 1992)

Genetic diversity, profit for the developing world

Professor Bob Thomas of Biotics Limited has been promoting a phytochemical screening programme since 1986 with the objective of cataloguing commercially exploitable genetic resources in the developing world. Thomas is keen to see a repatriation of wealth to the developing world on the basis of royalty payments. The company was founded in 1983 as a spin-off from Thomas' activities at the School of Chemistry and Molecular Sciences at the University of Sussex, UK. Laboratory services are supplied on a contract basis by the University.

The European Commission has given its backing to the Biotics programme, supporting a number of regional screening programmes. Already Professor Thomas has secured the interest and financial participation of a number of major companies and is supported by a University of Sussex programme which offers training facilities for scientists from the developing world.

Trainees might constitute the staff and managers of companies created in developing world countries with the objective of producing commercially interesting plant extracts. Biotics has supplied more than 2,000 samples of dried plants from Africa, Asia and Latin America to organizations concerned with the commercial screening of new biological activities. Biotics can support its commitment by offering a custom extraction service, which may tailor solvent extraction methods suitable for subsequent large-scale procedures. (Source: *BTE*, Vol. 9, No. 6, June 1992)

Biotechnology services for third world agriculture: new international initiatives

A real boom can be witnessed in the number of international initiatives that aim at providing biotechnological techniques and services to developing countries. Some of these focus on transferring specific techniques or on providing training; others have a broader scope, and will also provide advice on socio-economic and policy issues.

The Intermediate Biotechnology Service

Plans for setting up an Intermediate Biotechnology Service (IBS) are currently being discussed by the Taskforce on Biotechnology (BIOTASK) of the Consultative Group on International Agricultural Research (CGIAR). IBS can be helpful to developing countries to obtain an independent opinion about the feasibility and desirability of technical projects. The IBS will function as a clearing-house between the priorities of developing countries and expertise of advanced

institutes in the industrialized countries. The potential clients of the services are the various components of the national agricultural research systems in developing countries: universities, private institutes and non-governmental organizations.

Its primary functions will be to:

- Assist individual countries in the identification of priority problems;
- Provide services on socio-economic subjects, regulatory issues, intellectual property management, and information access;
- Assist developing countries in implementing biosafety regulations;
- Assist in the development of human resources and physical research capacity;
- Apply biotechnology in sustainable production systems and environment.

Based on the need assessments in developing countries, its main task will be to advise its clients on biotechnology. Once targets are identified, the entity will assist in all aspects involving the improvement of access to modern biotechnology.

The service will provide an "early warning system" for countries and commodities that might experience negative consequences of biotechnology, such as the replacement of certain tropical export commodities by products that are produced in industrialized countries. Also, strategies will be defined to overcome these negative consequences, including ways to maintain or improve the market share of natural products.

CAMBIA: enabling technology

The Centre for the Application of Molecular Biology to International Agriculture (CAMBIA) plans to become an independent, non-governmental, non-profit research institute. The primary objective of CAMBIA is to develop molecular biology-based methods that enable new experimental strategies to be applied in agriculture, which must be reliable and robust enough to be used by non-molecular biologists in the field. Local researchers and farmers will be actively involved in the research planning and application of the designed biotechnologies. Apart from the research division, CAMBIA intends to explore divisions for education and information. The organization is to set up a gene bank, which will serve as a centre of collection, assessment, archiving and free distribution of all DNA molecules (both vectors and genes) that are in the public domain. (Address: CAMBIA Organizational Office, Dr. R. Jefferson Dr. K. Wilson, Lawickse Allee 22, 6707 AG Wageningen, The Netherlands. Tel./Fax: (+31) 8370 26342.)

ISAAA: private-public cooperation

The International Service for the Acquisition of Agri-Biotechnological Applications (ISAAA, or IS triple A) is an international non-profit organization which aims at facilitating the acquisition and transfer of proprietary agricultural biotechnology applications from the private sector in the industrialized countries, for the benefit of the developing world.

The programme focuses on 10 countries (Indonesia, Malaysia, Philippines, Thailand, Brazil, Costa Rica, Mexico, Egypt, Kenya and Zimbabwe). ISAAA wants to provide "an honest broker service" that matches needs and appropriate proprietary technologies, and facilitates the formulation of proposals that meet the needs of both the technology recipient and the donor of the proprietary applications or "know-how". ISAAA works closely together with the Stockholm Environmental Institute to provide impartial advice on biosafety regulations to developing countries. (Address: ISAAA. Dr. C. James, Lisboa 27, Apartado Postal 6-641, 06600 Mexico, DF, Mexico. Tel.: (+52) 595 45395. Fax: (+52) 595 41069)

The Biofocus Foundation

The Biofocus Foundation is an initiative of the World Academy of Art and Science. The initiative consists of two parts that interact: *Biofocus* wants to identify target areas and potential entrepreneurs in order to stimulate the development of private enterprise in developing countries. It will assist in the preparation of business plans and submit these as viable projects to potential investors.

Bioresource will generate profit from the identified joint ventures, partnerships and direct investments. It will provide venture capital and will take shares in start-up companies and technology transfer bodies. As such, it will act as a hybrid between a profit-making company and an "ethical investment club".

Pre-projects are identified in the following areas:

- Microbial starter cultures;
- Coastal biotechnology (unconventional energy sources);
- Bagasse as a chemical and microbiological feedstock.

(Address: Biofocus. Prof. C. G. Hedén, The Old Observatory, Drottninggatan 120, 11360 Stockholm, Sweden. Tel.: (+46) 8 304930. Fax: (+46) 8 314620.)

FAO BRG: crop specific technology

The Plant Production and Protection Division of the Agriculture Department of the UN Food and

Agriculture Organization (FAO/AGP) plans to assist developing countries in the development of plant biotechnology. The FAO experience may be useful for a balanced addition of modern biotechnologies to crop improvement and crop protection research in developing countries.

FAO/AGP will therefore be expanded with a Biotechnology Research Group (BRG) and will conduct research, provide advice through an informal Biotechnology Advisory Panel, and provide training services through a fellowship programme. Research will also be contracted out to appropriate advanced laboratories. The BRG will be attached to an existing research group on cell and molecular biology (e.g. a university department) that would conduct research on specific problems relevant to developing countries' agriculture. (Address: FAO/AGP. Dr. E. Wagner, Via delle Terme di Caracalla, 00100 Rome, Italy. Tel.: (+39) 6 57971. Fax: (+39) 6 6799563.) (Source: *Biotechnology and Development Monitor*, No. 9, December 1991)

Biotechnology Advisory Commission

The Stockholm Environment Institute (SEI) works towards setting up an independent international Biotechnology Advisory Commission (BAC).

The BAC would provide impartial advice to relevant national bodies to assist developing countries in evaluating biotechnologies by verifying the applicability and safety of the technology and by suggesting how responsible and effective procedures might be instituted. More specifically, the mandate of the BAC would be to:

- Provide advice on the risks and benefits of proposed specific introductions of genetically engineered organisms;
- Advise on the potential risks and benefits that are expected to result from the introduction to a country of a specific recombinant product and on the appropriateness of the product to a particular aim identified nationally;
- Review specific agricultural biotechnology projects to advise on risks, particularly those related to biosafety, and provide impartial advice on the steps required for testing and product application, taking into account evolving international codes of conduct.

The BAC would consist of at least eight experts, recognized internationally in their respective fields, whose judgement would command respect in the absence of formal legal authority.

The BAC would initially provide its advisory services solely on an on-request basis to appropriate governmental agencies responsible for the testing and introduction of recombinant products in developing countries, but might in the future, with the permission of the Board, extend its mandate to accept requests from other entities.

The Executive Committee of the Board of SEI will be advised by an Expert Group Meeting (EGM) on how to proceed with the initiative. The EGM was scheduled to take place 28-29 April 1992.

For further information please contact: Mrs. Gertrude Wollin, SEI, Järntorget 84, Box 2142, 10314 Stockholm, Sweden. Tel.: (+46) 8 7230260. Fax: (+46) 8 7230348.

The BIOTOL project

This series of books has been developed through a collaboration between the Open Universiteit of the Netherlands and Thames Polytechnic to provide a whole library of advanced level flexible learning materials, including books, computer and video programmes. The series will be of particular value to those working in the chemical, pharmaceutical, health care, food and drinks, agriculture, and environmental, manufacturing and service industries. These industries will be increasingly faced with training problems as the use of biologically based techniques replaces or enhances chemical ones or indeed allows the development of products previously impossible.

The BIOTOL books may be studied privately, but specifically they provide a cost-effective major resource for in-house company training and are the basis for a wider range of courses (open, distance or traditional) from universities which, with practical and tutorial support, lead to recognized qualifications. There is a developing network of institutions throughout Europe to offer tutorial and practical support and courses based on BIOTOL both for those newly entering the field of biotechnology and for graduates looking for more advanced training. BIOTOL is for anyone wishing to know about and use the principles and techniques of modern biotechnology, whether they are technicians needing further education, new graduates wishing to extend their knowledge, mature staff faced with changing work or a new career, managers unfamiliar with the new technology or those returning to work after a career break.

The learning texts, written in an informal and friendly style, embody the best characteristics of both open and distance learning to provide a flexible resource for individuals, training organizations, polytechnics and universities, and professional bodies. The content of each book has been carefully worked out between

teachers and industry to lead students through a programme of work so that they may achieve clearly stated learning objectives. There are activities and exercises throughout the books, and self-assessment questions that allow students to check their own progress and receive any necessary remedial help.

The books within the series are modular, allowing students to select their own entry point depending on their knowledge and previous experience. These texts therefore remove the necessity for students to attend institution-based lectures at specific times and places, bringing a new freedom to study their chosen subject at the time they need and a pace and place to suit them. This same freedom is highly beneficial to industry since staff can receive training without spending significant periods away from the workplace attending lectures and courses, and without altering work patterns.

Further details may be obtained from: Open Universiteit, Valkenburgerweg 167, 6401 DL Heerlen, The Netherlands; and Thames Polytechnic, Avery Hill Road, Eltham, London SE9 2HB, United Kingdom.

The activities of the Foundation for Ecodevelopment (Stichting Mondiaal Alternatief) (MA)

The Foundation for Ecodevelopment (MA) is an internationally active, non-profit-making environmental non-governmental organization, a member of the European Environmental Bureau, Environment Liaison Centre and the International Federation on Organic Agricultural Movement. MA operates mainly through networking and cooperation with other environmental organizations.

In 1982 Penang, Malaysia, MA was one of the principal founders of the Pesticides Action Network, together with IOCU and IYF. In the seventies it succeeded - in close collaboration with many other environmental organizations like WWF and IYF - in pushing forward the "EC-Bird Directive" of 1979: a first example of "binding" international legislation with sanction-right.

Today the main areas of environmental work are: biotechnology, ecological agriculture, energy-saving cooking stove design, birds, chemical time bombs, agro-forestry and pesticides. Recently the dissemination of information by means of its publication series "ECOSCRIPTS" became once again one of the core activities of MA. The contents of the publications are mainly directed to the situation in developing countries. As communication in environmental networks is regarded as crucial, MA gives the highest priority to its Camelsnose Project: environmental E-mail- and BBS-system. The Camelsnose Project has become the very basis on which Mondiaal Alternatief is doing its environmental, non-profit business. Within the APC (Association for Progressive Communications) network links to APC countries are now being built.

Further details from: Mondiaal Alternatief, Foundation for Ecodevelopment, P.O. Box 151, 2130 AD Hoofddorp. Tel.: 02503-32305. Fax: 02503-41359. BBS: 02503-23609. E-mail: Greennet:ronaldg. Greennet:camelsnose. Fidonet 2:280/4.200.

B. COUNTRY NEWS

Belgium

Green light for micro-organism patent deposits

Belgium has recently become the twelfth country possessing an International Depositary Authority under the "Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Protection". Deposits of micro-organisms or plasmids are accepted in the four collections that constitute the "Belgian Coordinated Collections of Microorganisms, BCCM", as follows:

- Filamentous fungi and yeasts in either the biomedically oriented IHEM-collection in Brussels (Curator: Dr. N. Nolard) or the (agro-) industrially oriented MUCL-collection in Louvain-la-Neuve (Curator: Prof. Dr. G. L. Hennebert);
- Bacterial strains, including actinomycetes, but excluding pathogens belonging to a hazard group higher than group 2 of the "UK Advisory Committee on Dangerous Pathogens", at LMG in Ghent (Curator: Prof. Dr. K. Kersters);
- Plasmids as preparations of isolated DNA or in the form of a combination *E. coli* (host)/plasmids at LMBP (Director: Prof. Dr. W. Fiers, Curator: Prof. Dr. E. Remaut).

The BCCM guarantee the depositor full security and confidentiality through their compliance with the rules of the Budapest Treaty.

Detailed information can be obtained from: Jan de Brabandere, BCCM-coordinator, Belgian Science Policy Office, rue de la Science 8 Wetenschapsstraat, B-1040 Brussels. Tel.: (32) 22383520; Fax: (32) 22305912. (Source: *EBIS*, Vol. 2, No. 2, 1992)

Brazil

Orchid gene repository

Brazil's Distrito Federal happens to be an unusually rich locale for orchids - botanists have catalogued more than 250 species within its 5,000 square kilometres, compared to 350 species in the whole Amazon region. According to Rio's *Jornal do Brasil*, four scientists at the University of Brasilia are trying to preserve as many species as possible by creating Brazil's first germ plasm bank for ornamentals, at the orchidary at the Brasilia Botanical Garden. The group has obtained 250 million cruzeiros ($270,000) from the Government and will begin by building greenhouses to cultivate specimens they have, as well as additional ones they want to collect. (Source: *Science*, p. 1359, 13 March 1992)

Canada

Obstacles to Canadian biotechnology

Immigration policies that stop talented managers at the border, huge patent backlogs and limited intellectual property protection, faltering financing, inadequate research spending and hazy regulations. These are some of the obstacles currently hindering the development of biotechnology in Canada, according to "National Biotechnology Business Strategy: Capturing Competitive Advantage for Canada", a report issued by the National Biotechnology Advisory Committee (NBAC), a private group serving Industry, Science and Technology, Canada (ISTC, Ottawa, Ont.), a government department.

Although long on criticism, the report sees ample market opportunities within Canada, particularly in forestry, agriculture and waste management, and for exports as well. Failure to introduce new technologies would harm not only the nation's biotechnology companies, but its entire natural resource-based economy, warns the NBAC.

Canada is home to some 350 biotechnology companies, but only a small number have made public stock offerings, while Canada's biggest conglomerates, for their part, have been too slow to recognize the need for biotechnology research.

In the regulatory area, NBAC notes that inadequate staffing at two federal departments - Health and Welfare Canada and Agriculture Canada - have worsened delays in the approval process for biotechnology products. Not surprisingly, NBAC recommends adding staff committed to assessing such products.

The regulatory process could be further streamlined, NBAC argues, by accepting clinical or other data submitted in the United States or Europe. The report says a major barrier to the commercialization of biotechnology products is that new products are regulated on a case-by-case basis so that for each new product a new protocol is required.

Canada's patent system also needs harmonization with those of other nations, according to NBAC. Canadian patent law does not allow the deposit of multicellular organisms, which means in effect that no one can patent such organisms. The Cabinet has agreed to put forward a bill that would enable Canada to sign the Budapest Treaty, an international agreement recognizing the validity of such depositions in support of patent applications. That would bring Canada into harmony with Europe and the United States in this regard. NBAC also wants to see the elimination of Canada's compulsory-licensing law, under which a company that holds a patent for a drug but is not performing additional research or commercializing the product is required to license it to another company that will develop it. Canada also suffers from a huge patent backlog.

To bolster Canada's research infrastructure, NBAC wants to see the federal and provincial governments take over indirect costs of university research. Canada also needs a fermentation facility that meets good manufacturing practice (GMP) requirements, NBAC says.

NBAC's preferred solution would be for a consortium of private companies - with federal and provincial government aid - to establish such a facility.

NBAC also wants to see the Canadian Government take a more active role in encouraging the development of biotechnology products in forestry, waste treatment and agriculture, as well as pharmaceuticals, because much of the Canadian economy's strength lies in its natural resource-based industries. In particular, NBAC says that Forestry Canada, a federal department, should work with provincial governments to spur the adoption of genetically improved trees for forest regeneration. (Extracted from *Bio/Technology*, Vol. 10, February 1992)

Costa Rica

Costa Rica to capitalize and conserve its biological resources

Costa Rica, one of the world's smallest countries, has recently signed an agreement with the world's largest drugs company, Merck & Company, to identify and extract valuable substances from Costa Rican flora and fauna. These substances will be sold to the pharmaceutical industry and, possibly, help to conserve biological diversity. The agreement, signed in September 1991, was spearheaded by Costa Rica's National Biodiversity Institute (INBio), founded two

years ago in Heredia. According to a staff member, government officials from several tropical nations have consulted INBio on how to set up similar arrangements.

The agreement is the first of what INBio hopes will be several partnerships with drug companies. Merck will provide US$ 1 million over the next two years, along with seed money from American and European universities, foundations and governments. In return, Merck will acquire exclusive rights to screen for pharmaceuticals in any plant extracts collected for it by INBio. If the company develops a patentable product, whether from the natural compound or from a significant chemical modification of such a compound, INBio gets a large share (51 per cent) of the royalties. The royalties will be used as additional funds for INBio's current 10-year scheme to inventory the country's entire flora and fauna, the biggest biological project undertaken by any tropical country. This scheme is part of the country's large-scale conservation programme.

Negotiations with Merck took almost a year and several barriers had to be taken. Pharmaceutical companies need pure, uncontaminated samples. Field procedures, extraction techniques and reagents had to be standardized, and chemists had to be educated accordingly.

Biologists will now have to get permits and deliver samples of everything they collect from the forests to INBio. Still, there is the problem of enforcement, preventing collectors from sneaking out a promising product. Also, a valuable plant species in Costa Rica may occur in other tropical countries and be available to competitors of INBio's clients. (Source: *Biotechnology and Development Monitor*, No. 9, December 1991

Côte d'Ivoire

New research laboratory

The International Institute for Scientific Research for the Development of Africa (IISDA) is a newly established research centre located in the Côte d'Ivoire (Adiopodoumé). The Institute, supported by Canada, France and Côte d'Ivoire, now concentrates on the improvement of yam varieties and the treatment of malaria.

Various techniques, like meristem culture, diagnosis of the sanitary state of yam tubers through the use of enzymes, micro-tuber research and spontaneous mutation *in vitro* will be used to develop methods for rapid dissemination of improved yam varieties. Close collaboration is foreseen with the International Institute for Tropical Agriculture, Nigeria, which runs an extensive yam programme.

IIRSDA is setting up an epidemiology unit to study *anopheles* (larval and adult) and their relation with human beings. IIRSDA also plans to study anti-malarial drugs (new compounds, treatment regimes, networks to monitor drug resistance). Equipment is being obtained to make research possible on the culture of the *Plasmodium* parasite at different stages of its evolution. The Institute will link its malaria research programme to the Medical Research Centre of Franceville in Gabon.

Contact: Dr. Gaston Grenier (Director General), IIRSDA, BP V 51, Adiopodoumé (Abidjan), Côte d'Ivoire. Tel.: (+225) 414170, Fax: (+225) 456828. (Source: *Biotechnology and Development Monitor*, No. 10, March 1992)

Egypt

Training for Egyptian scientists

A collaborative project between the University of California at Davis and the Veterinary Serum and Vaccine Research Institute in Cairo is the latest effort by the US Agency for International Development (AID) to staunch the brain drain of African scientists. The agency has awarded $1.9 million for a research project on a vaccine for rinderpest that is meant to help in the transfer of recombinant DNA technology from the United States to Egypt.

During the next four years, 11 Egyptian scientists will study at Davis in the laboratory of Ethiopian virologist Tilahun Yilma who is director of the collaborative project. There they will learn how to make recombinant vaccine for rinderpest, which kills hundreds of thousands of cattle in Africa every year. Meanwhile, AID funds will be put to use building a modern molecular biology laboratory here which, it is hoped, will serve as a lure to attract the Egyptian scientists back home after training in California. (Source: *Nature*, Vol. 355, 16 January 1992)

Ethiopia

Ethiopian gene bank now coordinating African Genetic Resources Network

Ethiopia is one of the most treasured centres of genetic diversity. Very little is known, however, about the full extent of the genetic resources. Over the last two decades, the remaining resources in Ethiopia have been plagued by various ecological stresses, civil strife and human displacements to ecologically vulnerable areas.

In 1976, Ethiopia and West Germany made an arrangement to establish the Plant Genetic Resources Centre (PGRC) with the mandate to collect and study germ plasm in Ethiopia. To perform this task, PGRC received pledges of US$ 6.3 million to be used over a 10-year period. In this period, its *ex situ* accessions rose to 40,000 with a broad collection of food cereals, oil seeds, vegetables, spices and legumes.

In March 1990, the PGRC was selected to host the Regional Co-ordination Unit of the Genetic Resources Network of the African Ministerial Conference on Environment (AMCEN). The first meeting of the Genetic Resources Network of AMCEN took place at PGRC in Addis Ababa in September 1991. The meeting reviewed the work to be undertaken by PGRC as the Regional Coordinating Unit. The AMCEN network will assist the African countries to integrate conservation of its plants, animal and microbial resources with environmentally sound management and sustainable utilization of them. The activities of the Network will cover both *ex situ* and *in situ* conservation, and the establishment of data banks. (Source: *African Diversity*, No. 6, October 1991)

Biotechnology in Ethiopia

In spite of its richness in genetic resources, Ethiopia has not yet formulated a biotechnology policy. An Ethiopian Science and Technology Commission (ESTC) has been formed to address biotechnology within a framework that cuts across national research institutions. Most of these research institutions apply some forms of biotechnology.

The **Institute of Agricultural Research** (IAR) is Ethiopia's largest research centre. IAR is mainly engaged in applied research aiming at producing high-yielding varieties and developing sustainable farming systems. Biotechnology is used in only a few of the more than 1,000 IAR projects. Most of the biotechnology research is carried out in cooperation with the International Livestock Centre Africa (ILCA) and the Armauer Hansen Research Institute (AHRI). IAR also deals with issues of germ plasm exchange and biodiversity conservation.

The **National Research Institute of Health** (NRIH) was established in 1951 and largely addresses questions of public health and tropical diseases. NRIH has eight laboratories carrying out research on viral and bacterial diseases. Together with ILCA and AHRI, NRIH develops diagnostic methods and DNA probes to research leprosy, leishmaniasis and trypanosomiasis. The Institute receives funds from the Government, but a considerable part of its budget is raised by selling its own products and services.

The **Department of Biology of Addis Ababa University** (AAU) collects micro-organisms and uses plant tissue culture techniques to micropropagate forest trees to supply reforestation programmes.

The research activities of the **Plant Genetic Resources Centre** (PGRC) are now being extended to genetic engineering, plant cell and tissue culture. Currently, PGRC's tasks not only embrace seed exploration and collection, conservation, seed exchange and documentation, but also seed multiplication and utilization, as well as cytogenic research. PGRC applies tissue culture to rapidly propagate forest trees. The technique is also used for species that have proven to be difficult to cross-breed or do not generate seedlings under natural conditions. The Centre also carries out research on nitrogen-fixing bacteria to minimize the use of fertilizers.

Reference: Shibru Tedla and Costantinos Berhe (1991), Biotechnology in Ethiopia, Addis Ababa, Ethiopia. (Source: *Biotechnology and Development Monitor*, No. 9, December 1991)

European Community

EC national biotechnology associations launch new industry body

Organizations representing the interests of bioindustries in seven European countries took a major step towards increasing their visibility and accessibility in Europe by establishing a joint secretariat and administrative centre in Brussels. The new organization, called the European Secretariat of National BioIndustry Associations (ESNBA), has as its parent bodies the national bioindustry associations in Belgium, Denmark, France, Italy, the Netherlands, Spain and the United Kingdom. The seven organizations believe that Europe-wide coordination is vital for European companies to exploit the potential of emerging markets in the face of intense competition from biotechnology companies in the United States and Japan.

The executive council of ESNBA will meet every two months to set policy and coordinate operations. Activities of the central office will be carried out by its own staff under the supervision of an executive secretary appointed every six months from the parent bodies, on a rotational basis. The first to take up this position is Louis Da Gama, executive director of the UK BioIndustry Association.

Details from: ESNBA, Avenue Louis, 490 bte 9, B-1050 Brussels, Belgium, or on +32 2 646 37 03. Fax: +32 2 640 37 59.

National Bioindustry Associations

The founding national associations of the ESNBA are:

In the UK: The BioIndustry Association (BIA), 1 Queen Anne's Gate, London SW1H 9BT or on 071 222 2809. Contact: Mr. Louis Da Gama.

In Italy: Associazione Nationale per lo Sviluppo delle Biotechnologie (ASSOBIOTEC), Via Academia 33, 20131 Milano or on +39 2 636 2306. Contact: Mr. V. Lungagnani.

In the Netherlands: Nederlandse Industriele en Agrarische Biotechnologie Associatie (NIABA), Vlietweg 17, PO Box 185, 2260 AD Leidschendam or on +31 70 3270464. Contact: Mr. J. H. L. van Lissa.

In France: Organisation Nationale Interprofessionelle des Bioindustries (ORGANIBIO), rue St Dominique 28, 75007 Paris or on +33 1 47 530912. Contact: Mr. J. Lunel.

In Spain: Associacion de Bioindustrias, C/Bruc 72-74, 6 Planta, 080009 Barcelona or on +34 3 34874000. Contact: Mr. J. Guixer.

In Denmark: Foreningen af Bioteknologiske Industrier i Danmark (FBID), Novo Nordisk A/S, Novo Alle, 2880 Bagsvaerd or on +45 4444 8888. Contact: Dr. J. Mahler.

In Belgium: Belgian Bioindustry Association (BBA), rue de Crayer 6, Bruxelles 1050 or on +32 2 6460564. Contact: Mr. P. Crooy. (Source: *Biotechnology Bulletin*, January 1992)

SAGB bolsters ranks

SAGB, Europe's senior advisory group on biotechnology, has increased its membership from the original seven multinationals to 28 member companies, including seven of Europe's leading biotechnology enterprises. Extending the membership is expected to counter the criticism that SAGB was mostly concerned with issues affecting multinationals, a charge the organization has acknowledged.

By including input from the likes of BioEurope, British Biotechnology, Celltech and Plant Genetic Systems, SAGB now believes it has greater authority to lobby on behalf of the biotechnology-using industries.

SAGB says it is committed to promoting an awareness and understanding of modern biotechnology and its industrial applications. But while SAGB welcomed the endorsement by the Community of "its forward-looking policy on the establishment of European competitiveness in biotechnology", it is concerned about how protective towards the Common Agricultural Policy (CAP) certain elements within the Commission are and the impact such attitudes may have on the commercial development of biotechnology in Europe. (Source: *European Chemical News*, 3 February 1992)

Bioethics for STOA: S&T options assessment for the European Parliament

Bioethics in Europe: Preliminary report. This 100-page report by GAB (Gruppo di Attenzione sulle Biotechnologia), Milano, for the STOA (Science & Technology Options Assessment) of the European Parliament is the first step of the reporting phase of a project started at the end of 1990. The first, "preliminary consultation", phase set up contacts with national consultants in six EC member States; the second phase concerned the organization of the Milan Workshop on Bioethics, held in October 1991.

The preliminary report includes an introduction to bioethics and new biological technologies, followed by short scientific summaries of "direct ethical implications": mapping the human genome; prenatal diagnosis; genetic screening; human embryo research; foetal tissue transplants; *in vitro* fertilization; gene therapy; transgenic animals; patenting life forms; and of "indirect ethical implications": biological weapons; release of genetically modified organisms; contained use; animal hormones and consumer health; economics; agriculture; novel foods; pollution control; waste treatment and environmental management; patenting life forms.

The breadth of scope limits treatment of each topic to two or three pages, which specialists may find superficial, and in some cases biased. However, the report gives an overview of topics likely to be the object of public and political interest or concern. It includes useful lists of references - books, articles, reports, periodicals, associations. A further annex describes and lists the activities and documents of the Commission, the European Parliament and the Council of Europe.

A Final Report is foreseen during summer 1992, to complete the materials so far assembled by the addition of a synopsis and conclusions.

Some copies of the Preliminary Report are available from the STOA Secretariat (Mr. R. Holdsworth, Fax: (352) 43002418). (Source: *EBIS*, Vol. 2, No. 2, 1992)

Standards for biotechnology - Commission prepares mandate for CEN

The Commission's Communication on the Competitiveness of the Community's biotechnology industry of April 1991 (see *EBIS* 3, p. 3) called for "a clear and precise mandate" to be prepared by the Commission's services, in consultation with the European Committee for Standardisation (CEN) "in order that work in the field of standards may fully complement the Community's legislative work".

The objective of the mandate is twofold: to strengthen the competitiveness of the industrial activities based on biotechnology in the European Community and - by defining in concrete terms technical specifications, codes, methods, etc. - to provide the technical complement and support to the legislation. These standards for biotechnology will cover a wide area of application: operations with "traditional organisms and micro-organisms as well as with GMOs; areas subject to legislation as well as those which are not regulated.

The draft Commission mandate was discussed on several occasions by the Committee on Technical Regulations and Standards and received a favourable opinion from this Committee in April. It covers six areas related to biotechnology:

1. Laboratories for research, development and analysis: e.g. codes of good practice for laboratory operations, definition of the equipment needed according to the degree of hazard, methods for handling, testing and inactivating of waste, etc.;

2. Large-scale process and production: plant design, process design and operating procedures for large-scale fermentation and extraction processes;

3. Equipment: standard testing procedures for cleanability, sterilization and leak tightness; performance criteria for various types of equipment (e.g. HEPA filters, autoclaves, chromatography columns, etc.);

4. Modified organisms for use in the environment: methods for the detection and identification of GMOs, inserts and free DNA; methods for insert characterization;

5. Micro-organisms: e.g. examination of the various lists of plant and animal pathogens and of hosts and vectors which have been used to construct Group I organisms (reports to be produced);

6. Methods required for the implementation of quality control procedures.

Full details will be published in *EBIS* once the mandate is officially established between the Commission and CEN. (Source: *EBIS*, Vol. 2, No. 2, 1992)

Yeast genome project

European molecular biology will pass a major milestone. A team of researchers in laboratories scattered across the continent will publish the entire sequence of chromosome III of the yeast *Saccharomyces cerevisiae*. At 300,000 base pairs, it is the longest continuous stretch of DNA ever sequenced. The paper, to be published in *Nature*, is also a first of another kind: with 147 authors, it looks more like a publication in particle physics than molecular biology.

This dramatic example of biology-as-big-science comes courtesy of the European Community's (EC's) Yeast Programme, an ambitious effort to put *S. cerevisiae* on the map as the first eukaryotic organism to have its genome sequenced - all 16 chromosomes and 15 million base pairs. Thanks to a unique system of support possible only in the EC, the effort is like no other sequencing project in the world in the way it is organized, financed and carried out. But it does share at least one common feature with other genome projects: it has sparked a controversy over access to the data it generates.

The project is now onto its second phase, with one quarter of the sequences of chromosomes II and XI already completed. Eventually, EC-backed groups will directly sequence about half the genome. By 1995 half the genome will be sequenced and the job should be completed by 2002.

One feature that distinguishes the EC's part of the operation is the massive scale of the collaboration involved. The sequence was laboriously assembled by the work of 31 teams working at laboratories spread through 11 EC nations. Each laboratory agreed to sequence 10 kilobases of DNA handed out in the first two years of the project. Most of the 35 laboratories in the second phase are now trying to do 25 kilobases a year.

This "cottage industry" approach initially came in for criticism. Many scientists thought that it would prove impossible to coordinate and fund, but they had not reckoned with the fact that EC funding is specially designed to build large-scale network collaborations between nations. One reason the EC has made it work is the funding mechanism it has adopted. The EC pays 2 ECU per base pair - about double what is possible by the most efficient "factory sequencing". This means that the researchers get a little extra to spend on analysis of genes as well as grinding out sequences. Since there is a new gene every 2 kilobases in yeast's densely packed genome, that helps keep interest high and the project flying. (Source: *Science*, Vol. 256, 24 April 1992)

France

Biotechnology organization in France

The Government is supporting research in biotechnology through different research organizations. Average funding is in the range of FF 1.5 billion per year.

	Millions of FF
Centre National de la Recherche Scientifique (CNRS)	400
Institut National de la Recherche Agronomique (INRA)	350
Institut National de la Santé et de la Recherche Médicale (INSERM)	150
Institut Pasteur	150
Commissariat à l'Energie Atomique (CEA)	200

In France biotechnology is supported by a French National Programme coordinated by the Ministry of Research and Technology.

Public perception of biotechnology is positive in France and two commissions, one of genetic engineering and one of molecular biology, have been created:

- "Commission de Génie Génétique" attached to the Ministry of Research and Technology (décret du 11 mai 1989); and
- "Commission de Biologie Moléculaire" of the Ministry of Agriculture (décret du 4 novembre 1986).

The following areas constitute the French priorities in science:

- Molecular biology and recombinant DNA is a strong point of France but it is no longer an objective in itself within the biotechnology framework. The domain is a prerequisite for any biotechnology project;
- Microbiology is a very high priority in France. The field was very weak a few years ago which is quite paradoxical in the country of Pasteur. There is a need for further study into extremophiles, fungi, lactic bacteria, yeasts as well as microbial physiology;
- Protein engineering is now a priority with an important programme in the framework of CEA called "Protein 2000". The French CNRS is also developing an important programme "IMABIO";
- Plant biotechnology is progressing rapidly. INRA and seed companies (Limagrain, Rhône-Poulenc and Sanofi) are national leaders in the field;
- Immunology is a strong point in France in the Pasteur Institute, the Institute of Marseille and various companies;
- Engineering of fermentation, enzyme technology and downstream processing are progressing.

Generally speaking, researchers are educated in universities where courses are based on biological sciences. Engineers are educated in "Grandes Ecoles", where the focus is on process engineering and biological sciences. Both types of students can prepare a Ph.D in the field of biotechnology.

Private investment in biotechnology is of the order of FF 2 billion per year. There is an increasing number of joint programmes between companies (including European ones in the framework of EUREKA) and between companies and governmental research institutes. About 700 companies are linked to biotechnology but only 100 are driving forces in the field. Thirty companies are based on venture capital.

Application of biotechnology can be observed in the therapeutics (drugs) and prevention (diagnostic and vaccines). France is in a good position in prevention vaccines (Institut Mérieux), diagnostics, AIDS (Diagnostic Pasteur) and in an average position for drugs design. Sanofi, Rhône-Poulenc and Roussel Uclaf have the biggest research programmes and are the three major pharmaceutical companies.

Sales in 1989 were about FF 613 billion. The balance between exports and imports is FF 86 billion of which FF 51 billion came from raw materials. Exchanges with the EEC represent 65 per cent for exports and 67 per cent for imports.

Various branches of the classic food and fermentation industry, e.g. wine, cheese, alcohol and vinegar, have a great tradition in France. Companies active in the field of biotechnology are in:

Beverages	Pernod-Ricard, Moët-Hennessey, Louis Vuitton
Dairy	Bel, Sodima, ULN, Bongrain
Sugar	Beghin Say, Générale Sucrière, Sucre Union
Yeast	Lesaffre, Vitalevur
Baking	Pains Jacquet

Many small companies are very active in the field of additives and specific products. Two pharmaceutical companies, Sanofi and Rhône-Poulenc, are very active in diversification and are quite successful in the field of additives.

Some companies like Orsan, Eurolysine (amino acids), Roquette (modified starch, sorbitol, dextrose, HFCS ...), Rhône-Poulenc (xanthane) enjoy international success.

Sanofi and Robertet are involved in the development of biotechnology in the field of flavours and fragrances.

France is the second largest world producer of seeds. It is clear that biotechnology will considerably improve the technology of seed production.

Big chemical companies (Orsan, Sanofi, Rhône-Poulenc ...) and specific companies (Limagrain, Clause ...) are involved. The production of plantlets can be added (Delbard, Barberet et Blanc, Derlyl ...).

Two big companies, Lyonnaise des Eaux-Dumez and Générale des Eaux, are very active in biotechnology and are working on an international scale. Smaller companies, Valorga and Sobea, are more specialized.

France Embryon (Mérieux plus Sanofi) and ISA (Rhône-Mérieux) are exporting embryos. This field is promising due to the new application of biotechnology. (Source: *BFE*, Vol. 9, No. 1, January/February 1992)

Trade in blood

Two new guidelines issued by France's national ethics committee oppose the marketing of blood products and the patenting of the human genome.

Its advice on blood products, which can be marketed in Europe under an EC directive, warns that "after blood, all tissues and organs would be in danger of becoming items of trade". The guideline, issued just ahead of the Maastricht summit, said: "The success of European unification cannot come about unless ethical values are taken into account just as much as economic values".

The committee's ruling on the genome states that "all the information contained in the human genome belongs to the common heritage of humanity: it is the field of knowledge which cannot be covered by a monopoly".

The committee also called for the principle of open access to genome data banks to be preserved. (Source: *New Scientist*, 14 December 1991)

Bio-Avenir

Rhône-Poulenc (Paris) has announced more details of Bio-Avenir, its agreement with the French Government for collaborative biotechnology R&D. Among three areas already included in the programme is an atherosclerosis treatment, for which a cholesterol-dissolving lipoprotein has been isolated. Bio-Avenir is also investigating biological barriers in the search for products that can target drugs to specific sites. The third area involves identifying new herbicides and fungicides. About 500 scientists will be employed in the programme, which has a budget of F 1.61 billion ($290 million) and links the French major with several State organizations. Rhône-Poulenc currently spends F 5.5 billion/year on research. The Bio-Avenir partnership could be extended to include other companies "if they are prepared to inject the necessary resources"; discussions are already under way with Roussel-Uclaf (Paris). (Source: *Chemical Week*, 13 November 1991)

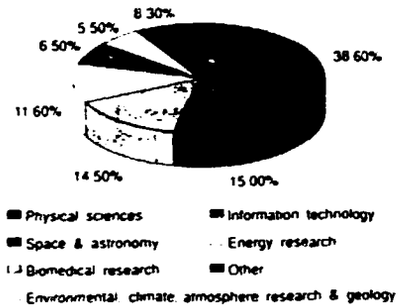
Germany

Funding shift from physics to biology

After a decade of generous support of large-scale physics programmes, the German Ministry of Research (Bundesministerium für Forschung und Technologie, BMFT) has decided to direct more of its funds into centres of excellence in the biological sciences. The change will relegate to a lower priority such new large items of equipment as accelerators.

The BMFT has adopted in principle the newly published recommendations of its advisory committee. In addition to a restructuring of Germany's research base, the committee has also suggested that industry should put more money into basic research, in particular such areas as chemistry.

Allocation of the 1990 BMFT budget for basic research
(total is DM3,170 million).



The report proposes new centres to specialize in genetics and neurobiology. They would be analogous to the National Cancer Centre in Heidelberg, the Ministry's major recipient of biomedical research funding and renowned for its work establishing a link between papillomavirus and cancer of the cervix. It also recommends greater support for existing groups at universities and research institutes working in such areas as AIDS and infectious diseases. (Source: *Nature*, Vol. 357, 21 May 1992)

India

Sericulture research and its impact in India

Other than China, India has the oldest tradition of sericultural practices and has the proud privilege of having a wide fauna of sericigenous insects and the flora of their food plants. India is the only country having the tradition of utilizing four silkworm varieties: mulberry

silkworm (*Bombysmori L.*), tasar (*Antheraea mylitta D.*), muga (*Antheraea assamensis*) and eri (*Philosamia cynthiaricini*). With the spread of sericulture from traditional areas to non-traditional zones and also to suit the changes realized in the country, it has become necessary to develop separate packages for low-cost rearing houses to rear adult silkworms and also rearing houses for chawki rearing. The tool of molecular biology has been of service to applied biology today, in a large way. The Government of India has considered sericulture as an appropriate field for developing biotechnological research infrastructure in India. Accordingly, the establishment of the laboratory of Seribiotech at Bangalore is in the offing. The Institute, at its primary stage, is expected to have three major laboratories: Molecular Genetic Laboratory, Laboratory for Vector Development and Laboratory for Germline Transformation. The future projection for seribiotechnological research includes short-term projects for development of diagnostic kits for diseases, biological control for uzi menace and also long-term projects on the improvement in the egg production potential of non-mulberry silkworm, development of better immunity response (higher germload) tolerance for the productive silkworm breeds, introgressing "resistance genes" in mulberry for controlling nematode infection and ultimately providing cost-effective *modus operandi* to improve the quality of the Indian silk and to help India in commensurating the deficit in the global silk production. (Source: *AIRD News*, May 1992)

Indonesia

High priority given to biotechnology

Indonesia is said to have the least developed biotechnology infrastructure of all the ASEAN countries but it is equally clear that the Indonesian Government has the will to overcome this, as it is actively building a biological sciences base.

Though inhibited by lack of manpower and funding, especially as the private sector is not sufficiently developed in biotechnology to assist, the Government has given biotechnology high priority. It has not yet put a master plan in place but is in the process of formulating such a plan.

The short-term strategy will be product development, to develop a biotechnology capability that will yield products of social and commercial value. Among the areas singled out are improved plant varieties, production of human vaccines, production of animal vaccines and diagnostics and production of Hepatitis B diagnostics.

There is already a Centre for Biotechnology set up by the Indonesian Institute of Science and various other institutions are tackling basic research. The Agency for Assessment and Application of Technology is looking into industrial applications, for example, single cell protein, ethanol production, industrial enzymes production and amino acid production. Specialist bodies such as the Plant Tissue Culture Laboratory and the Biotechnology Centre for Agriculture are all pushing research forward. Inter-University Centres for Biotechnology are researching agricultural, industrial and medical applications. (Source: *BIOTECH ASIA* '92)

Japan

MITI makes its move, cautiously

Japan's powerful Ministry of International Trade and Industry (MITI) is at last taking its first step into genome research.

The biochemical industry division within MITI will form a committee to coordinate various small projects that involve DNA analysis and consider a project to sequence the genomes of industrially useful micro-organisms. The eventual aim is a permanent government centre for DNA analysis.

MITI has hesitated to join genome research partly because the topic lies in the domain of other ministries but also because industry has shown little interest. Masahiro Hashimoto, deputy director of the biochemical division, emphasizes that the present initiative is meant to strengthen DNA analysis and is not a genome project. Hashimoto also admits that it may take a "long time" to persuade the Ministry of Finance that such a centre is needed.

The committee will try to coordinate three small MITI projects under the jurisdiction of the biochemical industry division: a project within Japan's huge fifth-generation computer project that is developing computer systems to handle the vast amounts of data arising from genome research; the application of nanotechnology, such as the scanning tunnelling microscope, to an analysis of DNA at a new interdisciplinary research centre in Tsukuba; and a small project within MITI's huge global environment research programme to isolate and develop photosynthetic micro-organisms to absorb carbon dioxide. (Source: *Nature*, Vol. 356, 19 March 1992)

Survey details Japan's use of bug sex pheromones

A survey by the National Institute of Sericulture and Entomological Science (NISEC), a research arm of the Ministry of Agriculture, Forestry and Fisheries (MAFF), has summarized the current state of research and commercial use of insect sex pheromones in Japan. According to the report, about 50 pheromones isolated from Japanese insect species have been structurally identified. Some 26 sex pheromones are now available as commercial formulations for use as insecticide substitutes. The report also points to the need in Japan

for further studies on the ecological impact of extensive use of sex pheromones as agrochemical agents. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 January 1992)

BTRAI to study plant defence systems

The Biooriented Technology Research Advancement Institute (BTRAI), a third sector organization formed jointly by the Ministry of Finance (MF) and the Ministry of Agriculture, Forestry and Fisheries (MAFF), said it will establish a research centre in Niigata Prefecture to study plant defence mechanisms. The centre will be used to conduct a seven-year project aimed at developing plant breeds with defence systems that provide endemic resistance to pathogens.

The long-term goal is to reduce the need for agrochemicals. Companies joining the study include Meiji Seika Kaisha Ltd., Idemitsu Kosan Co. Ltd. and Asahi Chemical Industry Co. Ltd. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

BTRAI to finance three biotechnology projects

The Biooriented Technology Research Advancement Institute (BTRAI), an organization established jointly by government and industry to support biotechnology-related research, said that it will finance three seven-year biotechnology projects starting in fiscal 1992. The projects include: (1) research to develop environmentally friendly methods to prevent plant disease, to be supported with 1 billion yen, (2) research using livestock to produce physiologically active compounds for use as drugs, to be financed with 1.4 billion yen, and (3) research to develop methods to artificially raise high-grade fish, to be financed with 1 billion yen.

Nine firms, including Meiji Seika Kaisha Co. Ltd., Asahi Chemical Industry Co. Ltd. and Idemitsu Kosan Co. Ltd., will participate in the plant disease project. Three firms, including Snow Brand Milk Products Co. Ltd. and Sankyo Co. Ltd., will join the livestock project, and four firms will participate in the fish-breeding study. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Staff centre to explore animal genomes

The Society for Techno-Innovation of Agriculture, Forestry & Fisheries (STAFF) has announced plans to construct a centre for advanced research in the fields of agriculture, forestry and fisheries. The new centre is to be constructed in Tsukuba. Studies at the centre will start with around 40 researchers. STAFF will use the centre largely for DNA-related studies. Initial research will aim to sequence the entire genome of rice, horses, cattle and pigs. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 April 1992)

Malaysia

Private sector biotechnology activity

Malaysia has a vested interest in agriculture and agribusiness and logically its biotechnology efforts are focused there. Progress in biotechnology is, in fact, driven by the large commodities industries, which are known to have developed very highly regarded research institutions.

Thus the palm oil, rubber, petrochemical, forestry and agriculture industries are all funding biotechnology research in some manner. The Palm Oil Research Institute, for example, does important work on tissue culture, molecular biology, enzymes and protoplasts. The Rubber Research Institute, the Malaysian Agricultural Research and Development Institute and the National University of Malaysia, which has a graduate programme in biochemical engineering, are all contributing to progress.

No master plan has been announced by the Malaysian Government as yet, though it acknowledges that biotechnology is a priority, and a body called The Intensification of Research in Priority Areas supervises a funding programme. Biotechnology is the responsibility of the National Council for Science and Technology Development.

The Malaysian strategy will most likely be to forge strategic alliances with suppliers of foreign technology, in order to overcome its lack of expertise and financing. A stronger enforcement of the patent system, too, is necessary if Malaysia is to move to the forefront in biotechnology. (Source: *BIOTECH ASIA '92*)

The Netherlands

Consumer and biotechnology foundation established

The Stichting Consument & Biotechnologie (Consumer and Biotechnology Foundation) was established by Dutch consumer organizations in January 1991, with financial support from the Dutch Ministry of Agriculture, Fisheries and Nature Protection. The Foundation aims to help Dutch consumer organizations to formulate their own policies on biotechnology.

Details from: T. Rullmann, Stichting Consument & Biotechnologie, Postbus 30500, 2500 GM Den Haag, the Netherlands. (Source: *Biotechnology Bulletin*, February 1992)

Portugal

College of Biotechnology, Porto

The Escola Superior de Biotecnologia (College of Biotechnology) was created in 1984, and evolved from an attempt of the Catholic University of Portugal to play a leading role in the development and modernization of the food and agro-industries of the country. Its premises are located in Porto (in the north of Portugal) and consist of 10,000 m^2 of covered area with classrooms, laboratories (with some of the most sophisticated analytical equipment) and a technological pavilion where the pilot plant equipment is located.

The College (ESB) includes, in addition to formal academic training, divisions of Extension and Research and Development. The faculty members have obtained academic and research training in respected institutions of such countries as the United States, Germany, the United Kingdom and France.

ESB offers B.Sc. degrees in Food Engineering, Microbiology and Environmental Engineering (five-year programmes), an M.Sc. degree in Food Science and Engineering (two-year programme), and post-graduate diplomas in Oenology and Brewing Science and Technology. Some of the courses are taught by lecturers from various countries and senior students are required to take a one-semester period of formal training abroad.

The Division of Extension provides services to the industry in the fields of food technology, food packaging, food quality, computing and information technologies, instrumentation and industrial electronics, and management and economics for agro-industries.

The R&D activities fall within seven major areas, namely food engineering, microbiology, enzyme technology, environmental engineering, plant biotechnology, bioprocess technology, and analytical chemistry. Most research projects in these areas focus on problems pertaining to food applications and are part of Ph.D formal programmes. The R&D activities are supported by national and international organizations such as the North Atlantic Treaty Organization, the Luso American Foundation for Development and various European Economic Community (EEC) programmes.

Support for the College has been obtained from 34 industrial partners, including not only national and multinational food and beverage companies, but also financial institutions and manufacturers of food equipment. These sponsors have assisted in the procurement of equipment and human resources.

For more information write to Prof. Augusto Medina, Escola Superior de Biotecnologia, Rua Dr. Antonio Bernardino de Almeida, 4200 Porto, Portugal (Fax: 351-2-400351). (Source: *International Newsletter (IFT)*, No. 38, February 1992)

Nigeria

New biotechnology initiatives at IITA

Researchers at the International Institute of Tropical Agriculture (IITA) are exploring the possibilities of applying advanced biotechnologies in their breeding work. Through its Biotechnology Research Unit, IITA is already active, mainly in tissue culture for germplasm conservation and the *in vitro* distribution of disease-free planting material of its mandate crops. In collaboration with institutes in Europe and the United States, IITA is currently entering the era of advanced biotechnology. Some highlights are:

- Interspecific crosses between cultivated cassava and wild relatives will be facilitated through embryo rescue and anther culture. Wild species of cassava have desirable characteristics for disease and insect resistance and low cyanide levels. From these crosses, IITA researchers have identified four spontaneous tetraploids and two triploids, while both cultivated cassava and its related wild species are diploids. Some triploids showed a 200 per cent yield increase compared to normal diploid cassava;

- In collaboration with the University of Leuven (Belgium), IITA is conducting research on the regeneration of cooking banana plants through somatic embryogenesis. To benefit from the potential of rDNA technology and somatic hybridization, the problem of poor regeneration of plants from single cells or protoplasts must be solved. An improved embryo culture protocol has been developed for the production of hybrid plantains. This is normally complicated by low seed germination rates. In soil, plantain seeds germinate at a rate of only about 1 per cent. Germination rates under the improved protocol range from 10-25 per cent;

- Wide crosses between African rice varieties (*Oryza glaberrima*, *O. longistaminata* and *O. barthii*) and *O. sativa* are being investigated. The African varieties have characteristics important for rice improvement in Africa, particularly immunity to the rice yellow mottle virus and resistance to the stemborer *Diopsis*;

- Several approaches are being examined to utilize biotechnology to solve the *Striga* problem associated with maize production in Africa. Projects are being developed with advanced laboratories in the United States and Europe already working with the molecular aspects of maize streak virus. The viral coat protein gene transfer approach may

be explored for all important African maize viruses;

- IITA's Virology Unit is collaborating with a laboratory of the US Department of Agriculture (USDA) at Beltsville, Maryland, in the production of monoclonal antibodies and cDNA probes for the detection of viruses affecting root and tuber crops. Another project has recently started for the production of monoclonal antibodies to detect viruses and their strains in food crops in various African countries. Because identification of viruses is more difficult than for other plant pathogens, many national institutes do not have equipment and other facilities to carry out work on virus identification. (Source: *Biotechnology and Development Monitor*, No. 10, March 1992)

Singapore

Biotechnology initiatives

Singapore has had a National Biotechnology Programme since 1988. Its priority is to develop a strong research and technology base and a significant step to realizing this was the setting up of the Institute of Molecular & Cell Biology (IMCB) at the National University of Singapore (NUS) in 1987.

University-industry collaboration is seen as an important plank in the programme. Initiatives such as the S$50 million trust fund set up by Glaxo for a joint research venture with the IMCB on degenerative brain disease is an example of the kind of collaboration Singapore welcomes.

The Biotechnology Competence Enhancement Programme supports a third aim, which is to establish a network of centres of excellence. The first centre, a Bioprocessing Technology Unit (BTU) was set up in 1990 to undertake multidisciplinary R&D in downstream bioprocessing and to engage in collaborative projects with industry. More recently the Food Biotechnology Centre was established to undertake applied food biotechnology research. The question of manpower availability in the South-East Asian countries is a vexed one. In Singapore the IMCB is the focal point for training R&D personnel. The NUS has integrated biotechnology-related subjects into its courses and the two polytechnics have begun specific diploma courses to train technicians.

Singapore is addressing an issue which could inhibit the growth of biotechnology in the region, by creating a legal framework to cover patents, good management practice and safe application of recombinant products.

A niche approach is adopted by the industry, capitalizing on existing strengths, infrastructure and Singapore's location. Therapeutics, diagnostics and diseases prevalent in the region; tropical plant biotechnology; tropical marine and aquatic biotechnology; bio-instrumentation and biotechnology-supporting service industries are areas which Singapore has targeted. To further boost the development of the industry, Singapore Bio-Innovations Pte Ltd. was set up by the Government to commercialize local inventions and innovations as well as invest in overseas projects of economic benefit to the country. It has invested in five projects since its inception in 1990. The Government welcomes investment by foreign biotechnology firms and has an attractive incentive package to encourage foreign companies to locate their projects in Singapore.

Public awareness of biotechnology is promoted through the Education and Public Awareness Programme which tackles it at all levels, from stimulating students' interest to educating industrialists on the potential of the industry. Retraining school teachers and setting up public exhibitions are just two of many projects undertaken under the scheme. (Source: *BIOTECH ASIA* '92)

Thailand

Agriculture-based applications

The growth of biotechnology in Thailand will undoubtedly be pegged to agricultural applications, with a focus on fermentation and feed industries. Indications are that in the long term Thailand will be active in the sectors of genetic engineering and pharmaceuticals.

Thailand has nominated biotechnology as a priority area and has a coordinated plan, much of which is in the hands of the National Centre for Genetic Engineering and Biotechnology. Thailand is particularly keen to promote R&D projects, though it is hampered by its limited manpower, its incomplete infrastructure and lack of cooperation between research institutions and the private sector.

As yet, Thailand has not moved on the issue of patents and protection of intellectual property. However, with agriculture so vital to its prosperity and agro-industry growing aggressively, Thailand is likely to put regulations in place in the foreseeable future. It has already identified 12 areas for emphasis in the fields of industry, agriculture, public health, environment and energy, and has set up a number of specialized laboratories in microbial engineering, plant genetic engineering, marine biotechnology and biochemical engineering. With funding from Japanese interests Thailand is also setting up an Agroindustrial Biotechnology Centre.

Over a four-year period, Thailand allocated US$49 million for R&D and for funding projects dealing with plant tissue culture and aquaculture, both of which are important to the Thai economy. (Source: *BIOTECH ASIA '92*)

Technology commercialization programme with the United States

Using its contract with the US National Research Council (NRC), the Thai Science and Technology Development Board (STDB) has launched the US-Thailand Commercialization of Science and Technology Program (UST-COST). The goal is to match United States and Thai companies in selected areas of technology: biotechnology, electronics and advanced materials.

COST has its origins in an experimental project directed towards technology transfer in biotechnology through commercial agreements with US biotechnology companies.

Principal partners were the US Agency for International Development (USAID), the Thai Science and Technology Development Board, the Maryland International Division (MID), the US National Research Council through its Board of Science and Technology for International Development (BOSTID), and BioTechnology International (BTI), a programme at the University of Maryland. USAID, STDB and MID provided the funding.

The project consisted of two distinct stages: an assessment by BTI of the needs of Thai industry and agriculture, and of the infrastructure in the biological sciences; and the identification and recruitment by MID of Maryland biotechnology companies that can provide the appropriate products and technologies. The State of Maryland had been chosen for this experimental project because of its existing biotechnology industry, its well-developed international trade office and the allocation of matching funds to the USAID contract.

The project began in late spring 1991. A trade mission of seven Maryland companies was organized. Upon their return to the United States, MID followed up on this initial series of contacts and tracked the progress of the negotiations.

Currently, there are over 20 agreements under consideration, with an estimated value of approximately $5 million. The proposed agreements can be categorized as follows: marketing/distribution, training, R&D, licensing/manufacturing and clinical trials. Many of the agreements being negotiated are in the biomedical sector.

Regarding the agricultural projects, two are related to aquaculture (i.e. fish growth hormone, shrimp feed), while another involves the development of new eucalyptus strains. The present status of these proposed agreements ranges from discussions to negotiations.

Given the initial success of the commercialization phase, the Thai Government showed its interest in expanding this project into other States.

Fernando Quezada, executive director of the Biotechnology Center of Excellence in Massachusetts, working along with BOSTID and STDB, visited Bangkok in 1991 to explore technology transfer agreements between US and Thai institutions, and to explore the commercial opportunities for Massachusetts biotechnology companies. (Source: *Genetic Engineering News*, January 1992)

United Kingdom

Aid for DNA research

A scheme aimed at boosting Britain's ability to profit from research on the human genome is to be launched.

The project will be run jointly by the Medical Research Council and the Centre for Exploitation of Science and Technology (CEST), an independent think-tank sponsored by industry and the British Government.

Huge amounts of public money are being spent on both sides of the Atlantic to unravel the mysteries of the human genome, to identify sequences of DNA and decipher which proteins they represent.

There is concern that Europe has not considered the implications of gene sequencing. For example, gene therapy could replace traditional drug treatment. One goal will be to establish practical ways to exploit and protect the findings of genome projects. A largely unrecognized problem is the task of making sense of the welter of data that is accumulating.

Already a large number of British, German, French, Swedish, Swiss and Italian companies have shown interest in supporting the project. (Extracted from *New Scientist*, 15 February 1992)

AIDS vaccine passes trials

British Bio-technology says that its AIDS vaccine, p24-VLP, has successfully passed through phase I clinical trials co-sponsored by the UK Medical Research Council. Detailed results could be announced at the international AIDS conference in Amsterdam later this year. Material for the phase II trials will now be made at the company's new biopharmaceutical plant in Oxford.

The vaccine is not designed to prevent HIV infection, but to halt the progression of AIDS in people who are already infected with the HIV virus. Genetic

engineering is used to produce the artificial virus-like particles (VLPs), which carry HIV proteins on their surfaces.

The recent study, supervised by Professor Jonathan Weber at St. Mary's Hospital in London, involved 16 healthy volunteers. The aim was to investigate the safety of p24-VLP, and to determine whether it could elicit cellular and antibody immune responses. The findings have yet to be fully reviewed, but public interest was sparked by a presentation of the unaudited results to the MRC. Phase II studies, which would focus on asymptomatic patients carrying the HIV virus, are now in the planning stage. (Source: *Chemistry & Industry*, 16 March 1992)

The National Institute for Biological Standards and Control (NIBSC)

The control of biological medicines and the standardization of biologies in general in the United Kingdom is primarily conducted in accordance with the following legislations:

- The Medicines Act 1968;
- The Biological Standards Act 1975;
- The National Biological Standards Board (Functions) Order 1976.

The overall aim of NIBSC is to safeguard and enhance public health through a programme of control, standardization and research related to biological substances used in the diagnosis, prevention and treatment of human disease. This is basically achieved by advising the Medical Control Agency (MCA) and others on scientific aspects of the licensing process and the results of the control tests of biologicals, and by processing and distributing national and international biological standards.

It is accepted that in technology generally, the UK weakness has been in the translation of discoveries into commercial products. Each of the progressive steps of biotechnology from discovery to "scale-up" involves new scientific questions relating to safety and product quality. Standardization and regulation is a critical step towards commercialization; it is also expensive and time-consuming. Clearly, it is critical for industry to have the use of working standards calibrated to recognized international standards. The Institute is a designated World Health Organization International Laboratory for Biological Standards. It is responsible for the world-wide availability of biological standards for the new development and testing biologicals.

NIBSC has steadily developed its expertise in biotechnology in the past decade. Its staff includes well-qualified scientists with special expertise in relevant disciplines, such as molecular biology, cell biology, protein chemistry and molecular genetics. In 1985 the Institute set up an informal Advisory Group on Recombinant DNA Technology and Monoclonal Antibodies. Among the activities of this multidisciplinary group are pre-licence discussions with manufacturers concerning standardization and control of biotechnological products. This approach clarifies for industry the circumstances under which a product may be refused a licence.

Once a biological medicine has received its initial licence, further product development will be inevitable. New products, processes and procedures are essential for business regeneration. Rapid changes in technology and strong competition continue to shorten product life cycles. NIBSC does not wait to respond to such changes - it anticipates them. This is a unique approach for a regulatory body and results in a sound relationship with the industry.

The Institute is a centre of scientific excellence occupying a unique position between basic research and commercialization. Membership of the BioIndustry Association provides an ideal forum for NIBSC to influence the processes of technology transfer and innovation in the biotechnology industry, especially that based in the United Kingdom. (Source: *BIA Bulletin* No. 19, December 1991)

UK okays BTG's growth hormone

UK officials have granted approval to a recombinant human growth hormone, developed by the US firm Bio-Technology General (BTG), allowing the drug to be used for the treatment of short stature.

The UK go-ahead follows recently announced technical approvals from Italy, the Netherlands and Spain and marketing approvals in Denmark and Luxembourg. Last June, the EC's Committee for Proprietary Medicinal Products approved its use for short stature indications.

Industry analysts put the estimated size of the European market for hGH treatment of short stature at some $250-275 million in 1991 and predict this could expand to $500-540 million by 1996. (Source: *European Chemical News*, 27 January 1992)

United States of America

FDA document on biotechnological foods

A soon-to-be-released Food and Drug Administration (FDA) document says that biotechnological foods will be treated no differently from other foods on the FDA's plate for review. The new policy, in keeping with the White House's recent desire to speed approval of biotechnological products, should simplify the regulatory process for tomatoes, potatoes and other edibles that have been genetically

engineered to - among other traits - taste better, ship more easily, or have built-in resistance to pests.

The draft document, being reviewed by the Public Health Service (PHS), says that the new biotechnological foods will be given special scrutiny only when they exhibit certain deleterious characteristics, such as increased levels of natural toxins or a tendency to form by-products not normally found in the food supply. A genetically engineered food would also trigger an FDA review if it could cause a new allergic reaction. For example, a tomato carrying a gene from a peanut plant to boost its protein might cause a reaction in people allergic to peanuts.

The new policy is on the fast track for approval at the PHS, the Office of Management and Budget, and the White House Council on Competitiveness. (Source: *Science*, Vol. 255, 27 March 1992)

US biotechnology policy

A recently re-chartered biotechnology advisory panel at the US Department of Agriculture (USDA) has criticized a proposal from Auburn University researchers who want to study transgenic catfish in outdoor ponds - but the panel still recommends limited approval for the experiment. Recent biotechnology policy statements from the Bush administration make the review panel's opinions appear even more perplexing, if not superfluous. Some critics say the panel and the transgenic fish appear to be swimming upstream towards a regulatory "never-never land".

After months of laborious rewriting, the USDA draft could be invalidated by the administration's recently completed "scope" policy statement. In essence, it says that biotechnology products are now considered "innocent until proven guilty", according to Alvin Young, director of the Office of Agricultural Biotechnology at the USDA. That seems to suggest that the transgenic fish just reviewed by the USDA panel were presented on a faulty pretext.

The committee seemed to think otherwise. Although it concluded that the experiments could go forward, several committee members voiced strong reservations about the quality of the proposed research, which involves catfish carrying a growth hormone gene from rainbow trout. Indeed, panel members and fisheries experts said that the tests could be considered safe for the environment only because elaborate precautions will be taken to confine the transgenic fish in a specialized facility. Moreover, it is still true that "no federal agency has formally declared jurisdiction over transgenic fish", says Marylin Cordle, another USDA official. (Source: *Chemistry and Industry*, 6 April 1992)

C. RESEARCH

Research on human genes

Tumour progression linked to gene mutation

Selective growth of cells containing mutations in a gene that encodes a tumour suppressor is responsible for the growth and increase in malignancy of brain tumours, according to Bert Vogelstein and co-workers at Johns Hopkins University's School of Medicine. Vogelstein's group, working in collaboration with researchers at three other institutions, showed that progression of non-invasive brain tumours (astrocytomas) to highly malignant brain tumours (gliobiastomas) was associated with mutations in what is known as the p53 gene. This gene, which encodes a tumour suppressor, is thought to act as a cellular "policeman", preventing anarchic growth by checking the activities of potentially cancer-causing oncogenes. Almost all glioblastoma cells contain p53 mutations, the researchers find. The same p53 mutation, however, is also present in a small number of astrocytoma cells. Tumours do not start out malignant, the researchers explain, but evolve from relatively benign, non-invasive growths. This work suggests "the progression of brain tumours is associated with a clonal expansion of cells that had previously acquired a mutation in the p53 gene, endowing them with a selective growth advantage" over other tumour cells, the scientists say. (Reprinted with permission from *Chemical and Engineering News*, 2 March 1992, p. 22. Copyright (1992) American Chemical Society)

Gene may give early clue to bowel cancer

Mutant genes from tumour cells can be detected in faeces. This discovery by American researchers holds out the hope of convenient and accurate diagnostic tests for bowel cancer.

Bowel cancer is the third most common cancer world wide. Although it can be cured by surgery, bowel cancer often goes undetected until intervention is no longer any use, and about half of all patients die.

Scientists have linked several mutant genes with an increased risk of cancers. One such gene, *ras*, is mutated in about half of all bowel tumours. Bert Vogelstein, David Sidransky and colleagues at Johns Hopkins University in Baltimore suspected that tumours carrying mutant forms of *ras* might shed cells into the colon. They reasoned that they might be able to detect the presence of a tumour by identifying DNA from these cells in faeces.

Vogelstein says that the team thought it unlikely that DNA would survive in detectable quantities. Nevertheless, they found some.

Vogelstein warns that it is too soon to know what proportion of tumours could be detected in this way. The method would have to be tested in a trial involving several thousand patients before doctors can use it.

The prospective test would only detect a proportion of all bowel tumours - perhaps a quarter. However, other genes involved in the disease may be identified in a similar way, using different gene probes. (Source: *New Scientist*, 11 April 1992)

Recombinant protein coaxes bone from cells destined to turn to muscle

Using a genetically engineered protein, a group of Johns Hopkins University researchers believe they have taken a first step to regenerating bone. So far, the scientists have experimented with the protein in animals only, but they hope to begin clinical trials this year.

The group, led by A. Hari Reddi, and Roger Khuri and Basem Koudsi of Washington University, St. Louis, recently reported results of the animal study in the *Journal of the American Medical Association*. The group injected the protein, osteogenin, into a flap of muscle in the legs of rats and clamped the muscle to a silicone mould. Later, the muscle had turned to real bone in the shape of the mould. Earlier, Reddi and his colleagues at the National Institute of Health (NIH), in collaboration with Genentech, Inc., So. San Francisco, isolated and purified osteogenin in 1988. The protein triggers stem cells, the undifferentiated precursor cells, to differentiate into bone cells, Reddi explained. Osteogenin is made up of 427 amino acids.

"We have proved, beyond a shadow of doubt, that this protein has the capacity to make stem cells differentiate toward the bone pathway", he said.

Dr. Reddi said the NIH has applied for a patent, which has not yet been issued. His group plans to soon file an application with the US Food and Drug Administration (FDA) for clinical trials with the protein. The first indication would be to repair non-union defects, which are stubborn fractures that will not heal. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 17 February 1992)

In record time, international team locates myotonic dystrophy gene

An international team of scientists have pinpointed the gene for myotonic dystrophy, just two weeks after announcing that they had found the region where the genetic defect was lurking. In time, the discovery of the abnormal, repetitive sequence on chromosome 19 will improve diagnosis and treatment for myotonic dystrophy, according to members of the international research team. The team consisted of scientists from Lawrence Livermore National Laboratory (LLNL), Livermore, California, the University of Ottawa, the University of Nijmegen in the Netherlands, the University of Wales and the Charing Cross and Westminster Medical School in London, Massachusetts Institute of Technology and Baylor College of Medicine.

Located on the end of a long arm of chromosome 19, the genetic defect is a repeating sequence of cytosine, thymine and guanine (CTG) bases. It appears between five and 27 times in people without myotonic dystrophy. It will repeat, however, 50 times or more in people who develop the disease. The scientists also discovered that the abnormal segment - an autosomal dominant gene - can get larger, causing more serious symptoms, as it is passed from one generation to the next.

The discovery of the gene gives scientists the capability to diagnose any individual with the disease. A diagnostic test is already available for Fragile X syndrome, a condition that is caused by a similar unstable gene ic region, this one a cytosine, guanine, guanine (CGG) repeat. Like myotonic dystrophy, the defect increases with succeeding generations. Scientists have already identified one protein product of the gene, an enzyme that is involved in opening and closing cellular channels and in signal transduction. Myotonic dystrophy, the most common form of muscular dystrophy, manifests itself through a range of debilitating and life-threatening symptoms. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 2 March 1992)

Zellweger syndrome gene found

Tadao Orii of the School of Medicine at Gifu University (Japan) and his team working in the Department of Paediatrics say they have identified the gene causing Zellweger syndrome, a hereditary disease that leads to death within the first year of life in most cases. Zellweger syndrome, also known as cerebrohepatorenal syndrome, is caused by the absence of peroxisomes that dissociate lipids within the body's cells. The condition leads to serious cerebral, hepatic and renal disfunction. The recessive autosomal gene is expressed in the population with an incidence of some one in one hundred thousand people.

Orii's team, in collaboration with researchers at the Meiji Institute of Health Science, isolated the PAF-1 gene that encodes the peroxisome membrane in normal cells. By comparing the base sequence with that of the same gene in Zellweger patients, the joint team found a termination codon at a site that should have encoded arginine at the 119th position in a 305-amino-acid molecule. As a result of the mutation, the peroxisome membrane is not formed. The team says the finding will provide the chance to develop a form of gene therapy to treat patients with the otherwise fatal disease. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Molecular machine springs trap for cancer cells

Molecules which can selectively invade cancer cells and tear their DNA to shreds have been made by chemists in California. They have been developed by modifying a natural anti-biotic of a type called an enediyne. Once inside a cancer cell, the modified molecules can change into destructive "free radicals" and kill the cell.

"Designer" enediynes have been made by K. C. Nicolaou and his colleagues at the Scripps Research Institute in La Jolla, California. Preliminary tests showed that they were more potent than anti-cancer drugs such as *cis*-platin, doxorubicin, bleomycin and taxol.

Nicolaou's team built an enediyne fragment into a molecule with other groups so that it can seek out cancer cells, invade them and once locked onto their DNA transform itself into a free radical. The chemists achieved this feat by attaching core chemical groups to the enediyne. The "detector" groups enable the chemists to track the molecule within a cell.

Natural enediynes were first identified in 1985 by Kiyoto Edo and his colleagues at Tohoku University Hospital, Japan. The first one, dubbed neocarzinostatin chromophore, was extracted from neocarzinostatin, a polypeptide known to have anti-cancer activity. In all these compounds, the enediyne unit is part of a much larger molecule. What Nicolaou's team has done is to stabilize the enediyne by anchoring it to a rigid double ring to which other groups are attached.

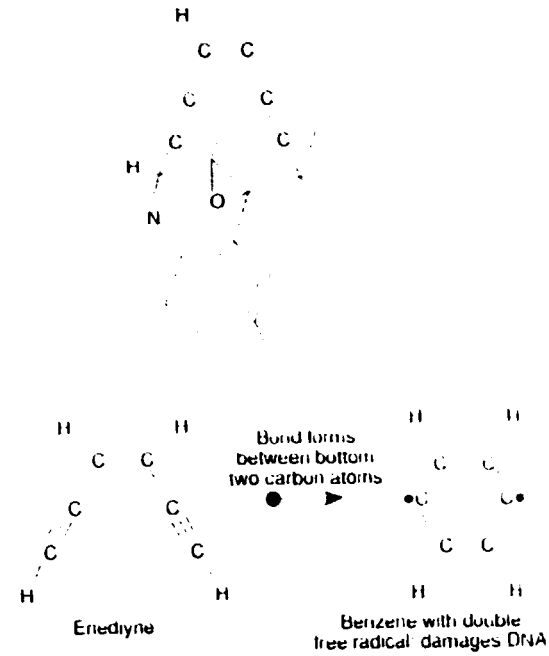
So far, the Californian chemists have made 11 designer enediynes, which are stable and inactive in neutral or acidic surroundings. Only under basic conditions do they become destructive.

In tests, the compounds tended to ignore normal cells, but they invaded tumour cells. A large percentage of the genetic material of invaded cells was damaged, even before death of the cell, showing that DNA was the primary target of the enediyne. The compounds were particularly effective against leukaemia. Tests on animals showed that the designer enediynes were less toxic than the natural enediynes.

Nicolaou tested his enediynes against a broad spectrum of cancers, and showed that changes to the group attached to the nitrogen atoms could increase the potency of the drug by up to 10,000 times.

In the anticancer molecule (see following diagram), an enediyne is anchored across a double ring. Inside a cell, this bridge breaks and the enediyne changes into a hydrogen-deficient benzene ring which has two free electrons. These destroy DNA.

Anti-cancer molecule primed to fire its deadly shot at DNA



(Source: *New Scientist*, 20 June 1992)

Deafness gene discovered

At the Boston University School of Medicine, researchers have discovered a gene, called HuP2, that plays a key role in congenital deafness. The BU team, led by Dr. Aubrey Milunsky, tracked six generations of a family in Brazil. Scientists examined the DNA of 60 family members - 26 of whom had Waardenburg syndrome, a rare disease that can cause deafness and other symptoms. The researchers also studied 50 unrelated people who did not have the disease.

The tests showed that the HuP2 gene had mutated in all of those who had the syndrome, while there was no defect in the gene of the unaffected family members or the control group. The discovery will enable scientists to make a precise diagnosis of the syndrome and provide genetic counselling. (Source: *Business Week*, 2 March 1992)

Sick policemen help to pinpoint arthritis gene

Salmonella food poisoning that struck hundreds of police officers during the Pope's visit to Canada in 1984 has given scientists valuable clues about a common type of arthritis called Reiter's syndrome.

Researchers had long suspected a link between bacterial infections and this type of arthritis in people with a genetic susceptibility to the disease.

Robert Inman, a rheumatologist at the University of Toronto, was able to pinpoint the gene responsible.

Inman and his colleagues sent a questionnaire to all the officers attending mass that day, and so identified those who developed pain and swelling in their joints after a bout of diarrhoea. A follow-up visit by one member of the research team confirmed that they were suffering from Reiter's syndrome, an arthritis that affects the joints of the hands and feet, the shoulders and the small of the back.

Comparing the group who had food poisoning but not arthritis with the group who had food poisoning and arthritis, the researchers pinpointed a gene called HLA-B 27 as the predisposing factor. Once they had discovered this, they could investigate the gene's role in the development of the disease.

The researchers hope they can transfer some of the lessons they have learnt from this isolated epidemic of Reiter's syndrome to other types of arthritis. Other studies have shown that Reiter's syndrome can be triggered by several types of bacteria and there are suspicions that other forms of arthritis have the same kind of link between genes and environmental triggers. (Source: *New Scientist*, 21 March 1992)

Engineered genes in grafted skin could be body's protein factory

Genetically modified skin grafts could as "factories for the body" for proteins that the body sometimes cannot make on its own. Researchers say that skin cells would be a safer way to introduce new genes into the body because the cells could be removed again if something goes wrong.

Elizabeth Fenjves, a professor of oral biology at the State University of New York at Stony Brook, has shown that human skin cells grown in culture can introduce new proteins into the bodies of mice. Fenjves grafted human skin cells onto a strain of hairless "nude mice", and later found a human protein called apolipoprotein E in the bloodstream of the mice. The protein was present as long as the graft was in place, and disappeared after it was removed. According to Fenjves, there could be several explanations for this. For the technique to work permanently, the genetic transformation must take place in stem cells that regenerate themselves, and not just surface cells that are simply replaced. Some proteins may have difficulty crossing the basement membrane that separates the epidermis from the lower dermis, and would not be able to get to the bloodstream.

Fenjves told a session at the AAAS that if these difficulties can be overcome, skin grafts could produce substances such as insulin for people whose bodies do not make enough. Because the skin is easily accessible and skin grafts can be reversed, they could become a safer way to carry out some forms of gene therapy.

Other researchers are now trying to add new genes to skin cells that could be used in grafts. Dusty Miller of the Fred Hutchinson Cancer Research Center in Seattle, Washington, has used a retrovirus to transfer a marker gene into skin cells taken from a dog, and successfully grafted them back. (Source: *New Scientist*, 22 February 1992)

Genetic link is discovered to high blood pressure

Scientists have discovered the gene behind a rare and severe form of high blood pressure that strikes early in life, often during childhood. Although doctors have long observed that high blood pressure can run in families, the research is the first to identify a gene that causes hypertension.

The finding offers researchers their first molecular grasp on a baffling disorder that is thought to result from a tangle of genetic flaws complicated by diet, obesity, lack of exercise and environmental factors. And though the gene detected is responsible for only a few thousand cases nation wide, researchers hope it will lead them to other genes that play a role in high blood pressure and allow them to better focus treatments for the disease. (Source: *International Herald Tribune*, 23 January 1992)

Overactive T-cells may cause asthma

The prospects of a new approach to treating asthma have improved sharply. British researchers have provided the strongest evidence that asthma results when cells in the immune system mistakenly become overactive. More importantly, it opens the way for trials of a new generation of asthma drugs which block these cells.

The latest work has been done by Barry Kay and Andrew Alexander at the National Heart and Lung Institute at the Royal Brompton Hospital in London, and Neil Barnes at the London Chest Hospital. The team, along with others, have suspected for several years that the T-helper cells of the immune system play a central role in asthma. When activated, these cells produce a range of "messenger" proteins called cytokines, which in turn trigger a set of events that lead to the constriction of the airways.

The new findings appear to confirm that activated T-helper cells are the central culprits. The researchers performed a clinical trial of the drug cyclosporin in 33 patients with severe, chronic asthma. Normally, cyclosporin is used to prevent the rejection of transplanted organs. It is thought to work by preventing the process that activates T-helper cells, although it also inhibits some other types of cell. (Extracted from *New Scientist*, 15 February 1992)

Gene linked to early hardening of arteries

Sausages and cigarettes may cause arteries to clog, but a gene carried by 24 per cent of the population also shares some of the blame. Scientists in Texas said they had cracked the code for one such gene, and their results could help in predicting who is at risk.

The gene is one of several that produce apolipoproteins, components of the lipoproteins that shuttle cholesterol through the bloodstream and to the liver. Called *APOe*, the gene comes in several versions, or alleles. The three most common are called *E2*, *E3* and *E4*.

Everyone has two versions of the gene, one inherited from each parent. Fifty-seven per cent of the population is *E3 / E3*, 24 per cent is *E3 / E4*, and 13 per cent is *E2 / E3*; other combinations are rare.

James Hixson, a geneticist at the South-west Foundation for Biomedical Research in San Antonio, Texas, examined DNA from 683 men, aged between 15 and 34, who had died in accidents. He then compared the allele type with maps of the walls of major arteries removed during autopsy.

Every subject had some plaque covering the interior walls of their abdominal artery. But those with the genetic combination *E3 / E4* showed the most damage; 32 per cent of the artery was covered. Those with the combination *E3 / E3* were somewhat better off, with 29 per cent covered, while the *E2 / E3* group had 23 per cent damage.

The differences may seem small, says Hixson, but they are statistically significant considering how young the subjects were. The subjects with the *E2 / E3* allele had almost 40 per cent less damage than the *E3 / E4* group, suggesting that the *E2* allele has a positive effect on clearing cholesterol from the bloodstream.

Other studies have linked the *E4* allele to a higher risk of heart attack. In addition, Hixson found that the *E2* allele is associated with lower blood levels of low-density lipoprotein, believed to be the more harmful form of cholesterol.

Screening young people for a genetic predisposition to atherosclerosis would pinpoint those who especially need to watch their diet and behaviour. Some 20 genes have already been implicated in the body's cholesterol path-way, but Frederick Cornhill of Ohio State University says Hixson's research is the first to show that a single gene is probably as important as any of the traditional behavioural causes of the disease. (Source: *New Scientist*, 25 January 1992)

Gene therapy promises cure for cystic fibrosis

Prospects of a cure for cystic fibrosis improved dramatically with the announcement that researchers have successfully introduced a corrective gene into the lungs of experimental rats.

Ronald Crystal, leader of the research team, and his colleagues at the Heart, Lung and Blood Institute in Bethesda, Maryland have shown that they are able to produce a healthy version of the cystic fibrosis gene into the lung tissue of rats, and that the cells then produce the key protein normally.

The researchers have not "cured" the disease because no experimental animal actually suffers from cystic fibrosis, but the prospects of a cure in humans are considered very good. Cystic fibrosis is the commonest fatal hereditary disease.

Trials in human sufferers are still some time off. "Before we can go ahead with human therapy, we have to demonstrate safety", says Crystal. Safety tests are expected to take about a year.

The genetic defect that causes cystic fibrosis was discovered in 1989, the result of collaboration between American and Canadian research teams. The faulty gene controls production of a protein that regulates the flow of chloride ions across cell membranes. The cells that line the lungs are particularly sensitive to a defect in this gene, and the result is a build-up of mucus. This encourages frequent bacterial infections, which progressively destroy lung tissue. Most sufferers die before they reach 30.

The researchers took samples of an adenovirus which infects lung cells and causes cold symptoms, and debilitated it by removing its means of replication. They then inserted a healthy cystic fibrosis gene into the virus and introduced it into the rat's lungs by a nasal spray. As the genetically engineered virus makes its way to the cells' nuclei it breaks up and releases the cystic fibrosis gene. In a way that the researchers do not yet understand, the cells' machinery then processes the gene normally and produces copious quantities of the chloride-regulating protein.

Because mucous membrane cells of the lung are constantly shed, and have a lifespan of just a few weeks, the introduced genes are gradually lost. Crystal and his colleagues found that after six weeks the level of introduced cystic fibrosis gene falls to about 40 per cent of the original. This means that the effect wears off and sufferers would have to have regular treatment. But because the protein is effective at extremely low levels, Crystal guesses that repeat treatments will be needed only every few months.

The safety issues under investigation include the effects of higher than normal levels of the chloride-regulating protein, the potential for the cystic fibrosis gene to get into other cells, including germ cells, and prospects of infection from a treated patient to other people. Crystal and his colleagues are confident that they will not encounter any major problems. (Source: *New Scientist*, 18 January 1992)

The parasite that exploits the immune system

A parasite that infects 200 million people around the world, and leads to the disease schistosomiasis, relies on its host's immune response to survive. This discovery, by American researchers, offers clues to treatments that might overcome the parasite. It could also lead to therapies for other common diseases, including TB and syphilis.

Schistosomiasis, or bilharzia, is caused by flatworms of the genus *Schistosoma*. The worms, which are transmitted by infected snails, are common in parts of Africa and Latin America. Water development projects, such as the building of dams and irrigation schemes, appear to favour the parasites. They lay their eggs in human intestines, causing inflammation and the build-up of nodules known as granulomas.

Granulomas do as much damage as the parasite itself. They develop because the T-cells of the human immune system attempt to defend the body against the parasite, causing inflammation. Until now, immunologists had thought that a complex mix of immune reactions was responsible, involving different T-cell types and cytokines, the messenger proteins of the immune system.

Now, however, James McKerrow, Payman Amiri and their colleagues at the University of California at San Francisco have shown that one cytokine alone is responsible for granuloma formation. More surprisingly, they have discovered that female schistosomes will only lay their eggs when the cytokine is present.

The team studied specially bred mice known as SCID mice which have no immune system: they lack T- and B-cells so cannot produce cells and antibodies against infection. When infected with *Schistosoma mansoni*, these mice did not form granulomas and the number of eggs the worms produced was sharply reduced.

McKerrow and his colleagues then returned part of the immune system of the infected SCID mice by injecting T-helper cells known as Th2 cells. These cells produce a specific set of cytokines. By blocking each cytokine in turn with a specific antibody and observing what effect this had on granuloma formation, the team narrowed the search to one cytokine, TNF alpha.

Next, the team injected schistosome-infected SCID mice with purified TNF alpha alone. Granulomas formed around the eggs: the more TNF, the more granulomas. Also, the greater the dose of TNF, the more eggs the females laid. When the researchers cultured adult pairs of worms in the presence of varying amounts of TNF, egg-laying increased in line with the dose (*Nature*, 16 April, p. 604).

The findings demonstrate the close relationship between parasite and host.

The research also has "profound implications" for therapy, he says. Blocking TNF with a specific antibody should stop the damaging granulomas and the laying of eggs. Another group of researchers on the same track is already testing an anti-TNF antibody in humans.

McKerrow is more cautious, because granulomas turn out to have a purpose. While they certainly cause long-term disease, they may prevent a more acute disease. Schistosome eggs secrete a substance, as yet unidentified, that is highly toxic to the liver. The granulomas prevent the toxin from escaping, suggesting the immune system has evolved this form of partial protection. McKerrow is working with a team at the University of Wales to identify the toxin and make a specific antibody to block it. The two treatments could then be tested together, he hopes.

Granulomas are an important cause of the damage caused in TB and syphilis, among other infections. Anti-TNF therapy might also help these diseases. (Source: *New Scientist*, 25 April 1992)

Gene gives clue to how cancers grow

One of the many contributory factors in cancers could be the gene p53, according to American researchers.

Mutated p53 genes are found in cancers of the colon, lung, breast and ovary. Scott Kern and colleagues at the Johns Hopkins University in Baltimore have shown that when the protein made by a healthy p53 gene attaches to its binding site on DNA, it can trigger a neighbouring gene to make its own protein. As mutant p53 genes are found in human cancers, the result adds to evidence that p53 is crucial to tumour growth.

Scientists believe that p53 triggers genes to produce proteins which in turn suppress cell growth. When the p53 gene mutates, cells grow out of control.

The researchers spliced the DNA binding site for normal p53 next to the gene that codes for chloramphenicol acetyl transferase (CAT), an easily detected protein. They then inserted this sequence into human colorectal cancer cells.

Normal p53 protein activated the CAT gene, and the more p53 binding sites added in a row, the more CAT was produced. But variants of p53 produced by tumours could not trigger the CAT gene to make proteins. (Source: *New Scientist*, 16 May 1992)

Cancer drugs target cells that other treatments miss

Clinical trials are beginning of three new drugs which target cancer cells that conventional chemotherapy and radiotherapy cannot reach. The agents attack cells which are hypoxic, that is, cells with a lower-than-usual level of oxygen.

If the agents prove acceptable they will probably go into further trials in a wide range of tumour types including breast, bowel, cervix, lung and brain.

The structure and function of blood vessels in tumours tend to be disorganized. As a result, some cells have poor blood supply which leads to a lack of oxygen. These hypoxic cells are doubly protected against existing cancer therapies. Cytoxic drugs reach them only in low concentrations. And, because of their low level of oxygen and nutrients, hypoxic cells have a relatively inactive metabolism. This means they may not be undergoing the cellular processes that chemotherapy disrupts. Radiotherapy is also ineffective against hypoxic cells because oxygen is necessary to damage DNA permanently. The new agents are different. Researchers have shown that the agents are converted into their active, cell-killing form in a reducing, or oxygen-poor, environment.

One is a quinone known as EO9. Research on EO9 has been conducted by British, European and American cancer research organizations independently of any single pharmaceutical firm. The drug will soon begin trials in Rotterdam in the Netherlands.

The other two drugs are Sterling Winthrop's compound WIN 59075 which is starting trials in Glasgow and RB 6145, a compound developed by Britain's Medical Research Council's Radiobiology Unit, which is based at Harwell.

The new drugs are converted to their active form by cell enzymes; EO9 is reduced by DT-diaphorase. The conversion can happen in any cell where the right enzymes are found, but in the normal, oxygen-rich environment the active form is quickly oxidized back to the less toxic parent drug. Only in hypoxic conditions is the active form stable. Once in a hypoxic cell, the drugs destroy DNA, chopping it up into small pieces.

The drugs are presently being tested only for safety and to assess the right dosage. (Source: *New Scientist*, 16 May 1992)

A critical gene is identified in cell life

Searching amid the twisted wreckage of chromosomes found at the heart of nearly all human cancer cells, scientists have identified a handful of molecular aberrations that seem to be, not the incidental debris that comes with malignant transformation, but the fundamental defects that helped spawn the cancer in the first place.

Among the most dangerous and widespread mutations they have found is one that disrupts a gene with the name of myc. Whether in tumours of the breast, brain, bladder, blood, lung, colon or other body parts, myc has been seen.

The gene is so frequently disturbed in cancer tissue, and in its normal guise it bears so many trade marks of being critical to the life and upkeep of all body cells, that researchers cannot help but call it McGene. Everywhere they look, there it is, the myc gene. After nearly two decades of alternately dabbling with the gene and then abandoning it as too hard to decipher, researchers have made a series of breakthrough discoveries that are bringing this extremely important molecule into focus. Many of the results are basic revelations about how a cell knows when to divide, when to mature, and on occasion, when to commit suicide for the good of the body.

The preliminary findings have implications for cancer treatment, possibly in the near future. Scientists believe the gene may prove valuable as a prognostic tool for breast cancer, as a way of distinguishing between early breast tumours that can probably be treated successfully through simple surgery, and highly aggressive cancers that are likely to recur and thus should be treated with the most blistering chemotherapy available.

In a study published in the journal *Cancer Research*, scientists from the Netherlands said that women whose breast cancer cells harboured an abnormally high number of myc genes were far more likely to suffer a recurrence after surgery than women whose malignancies lacked signs of aberrant myc amplification.

Test-tube and mouse experiments also suggest that of the many genetic flaws in the average cancer cell, the myc defect is so nasty that its elimination alone may be enough to cure or at least tame a substantial fraction of tumours.

Using medications that have been around since the 1960s, Dr. Geoffrey M. Wahl of the Salk Institute in La Jolla, California, and Dr. Daniel D. Von Hoff of the University of Texas Health Science Center in San Antonio have managed to correct myc defects in cultured cancer cells. The drugs work by encouraging the cells to eject their excess copies of the gene, and once

those perilously amplified genes are eliminated, the cells revert to a seemingly non-cancerous state.

The researchers are now exploring whether this approach has any medical value. They have begun clinical trials for the treatment of advanced ovarian cancer through the use of a drug called hydroxyurea, which combats abnormal gene amplification. They suspect that the myc protein is a kind of toggle switch, sitting at the junction of two options an active cell must choose between: to proliferate with aerobic vigour, or to differentiate into a more sedentary state as a mature member of the lung, breast or other organ.

The myc protein seems to be necessary for a cell to begin dividing and to keep dividing, and it must be firmly silenced before any cell can mature into its final stage.

Biologists have also been delighted to discover the partner of the myc protein, another enzyme with the name max, which myc must embrace before it can do anything in the cell. The identification of max has allowed scientists to begin figuring out precisely how myc encourages an act like cell division.

But of greatest surprise to those in the myc field, biologists at the Imperial Cancer Research Fund in London lately have found still another task of the myc protein, one that on first consideration flouts common sense. Dr. Gerard I. Evan and his colleagues discovered that the myc protein, famed as a master of cell proliferation, can also initiate cell death. When cells growing in a test tube are stripped of their nutrients, the myc protein starts a violent chain reaction called apoptosis, culminating in the cells' demise.

Dr. Evan proposes that, while the idea of linking cell death to cell growth seems counter-intuitive, evolution had good reason to make the connection. Cells must be able to divide in a healthy body to replenish lost tissue, he said, but should that division encounter any sort of difficulty, like a disturbance in surrounding biochemical signals, and the possibility of renegade growth arises, the safest course for the cell to take is to set a suicide programme in motion. (Source: *International Herald Tribune*, 7 May 1992)

UV radiation tied to human cell mutation

Scientists for the first time have clearly demonstrated that a mutation in human tumour tissue is tied to ultraviolet radiation.

Led by investigators at the Yale School of Medicine (New Haven, CT), a research group has detected the genetic damage that results from excessive sun exposure. They found that in patients with squamous-cell carcinoma, ultraviolet rays disable the p53 gene which controls cell growth and has been linked to various cancers.

Until now, there was little evidence about what causes the gene to mutate, but scientists suspected that environmental factors, such as chemical carcinogens, sunlight or radiation, were responsible.

There are data suggesting that people who inherit a mutated version of p53 (or other tumour suppressor genes) are more susceptible to cancer. But the majority of scientists believe that most cancers develop when these genes are deactivated through a spontaneous mutation during cell division or from exposure to a carcinogen. Now, they have proof of this connection.

Results show for the first time how a common human carcinogen works, says Douglas E. Brash, Ph.D., a biophysicist who is assistant professor of therapeutic radiology at Yale University School of Medicine and a member of the Yale Comprehensive Cancer Center. He led the eight-member, multidisciplinary research team from Yale, Massachusetts General Hospital (Boston), Dermatopathology Associates of New York in New Rochelle, and University Hospital in Uppsala, Sweden.

The researchers examined the tumour tissue of 24 people with squamous-cell carcinoma. In 58 per cent of the patients, the p53 gene bore a specific mutation that is typical of the kind that occurs when genetic material is exposed to ultraviolet light.

In laboratory studies, ultraviolet light alters DNA in two ways, Dr. Brash explains. First, it links neighbouring thymine and cytosine bases. Mutations occur where two of these cytosine-thymine pairs are adjacent. When cells divide and copy their damaged genetic material, the cells' enzymes can mistakenly substitute one or both bases with a different base.

The second unusual consequence of ultraviolet light, and the tell-tale sign of a mutation caused by this type of radiation, is that when the cell repairs the damage, one particular base substitution predominates - in this case, where two neighbouring DNA bases are cytosine, they are both mutated to thymine.

When this occurs, the gene manufactures a defective protein that destroys the cell's growth regulatory system. The newly produced cell and all subsequently produced cells will contain this same mutation. "Thus, a tumour could show the footprints of sun damage that occurred decades ago", Dr. Brash points out.

Scientists have found disturbances in the p53 gene in practically every type of tumour they have examined, including malignancies of the colon, breast, lung, kidney and brain. They believe a disruption in p53 may be a critical step in cancerous transformation, though they think several mutations are involved in full-blown malignancies. In the case of skin cancer, Dr. Brash suspects that the other changes involve additional genes or sunlight damage to a person's immune system.

Dr. Brash and his team are now planning to examine melanoma and basal cell tumours to see if they also bear p53 damage by ultraviolet rays. Scientists believe melanoma may result from blistering sunburns that occur during adolescence.

Researchers hope to use the findings to develop new treatments for skin cancer. For all other types of cancers, treatment must be administered systemically. For skin cancer, since the lesions are accessible, a non-invasive, DNA-directed therapy may be possible, explains David Leffell, M.D., chief of dermatological surgery at Yale. "It may become possible to inject patients with the corrected genetic pattern to induce a clone of cells that would divide in a normal fashion", he says, though he stresses this application is probably years away.

Moreover, with a knowledge of the precise genetic defect, if scientists can determine which ultraviolet wavelengths are responsible for causing the mutation, it might be possible to develop specific sunscreens to block these particular wavelengths, Dr. Leffell says. (Source: Genetic Engineering News, January 1992)

Potential treatment for sepsis developed

A natural antibiotic could be a potential treatment for sepsis. The agent, called bactericidal/permeability increasing protein, or BPI, exists naturally in human white blood cells. It holds promise in the treatment of sepsis because it specifically attacks and kills gram-negative bacteria, and also neutralizes lipopolysaccharide (LPS), the toxic part of endotoxin (a poison released by gram-negative bacteria).

The research was conducted by Drs. Peter Elsbach and Jerrold Weiss of New York University Medical Center (New York, NY), in collaboration with scientists from Xoma Corp. in Berkeley, CA. The scientists used the cloned BPI gene to produce larger quantities of the protein and derivative fragments containing the active regions.

In laboratory experiments, a fragment of natural BPI when added to whole blood, both killed bacteria and stopped the self-destructive host responses triggered by endotoxin.

In gram-negative sepsis, endotoxin stimulates the body's release of a variety of substances including tumour necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8). The release of these substances leads to clinical problems, including kidney and liver dysfunction, blood coagulation abnormalities, respiratory distress and circulatory insufficiency.

The scientists have shown that a fragment of the BPI molecule inhibits the release of TNF, IL-6 and IL-8 from white blood cells. (Source: *Genetic Engineering News*, January 1992)

Novel function discovered for cystic fibrosis gene

In the two years since the cloning of the cystic fibrosis (CF) gene, evidence has mounted that the gene produces a protein that ushers chloride ions out of cells. Many researchers have come to believe that all of the symptoms of the deadly disease derive from the mutant protein's failure to perform this basic function. But a paper in *Science* by Neil Bradbury, Kevin Kirk, Robert Bridges and their colleagues at the University of Alabama suggests that the CF protein controls other cellular processes besides chloride secretion.

Their group found that the CF protein (known to researchers as the CFTR, for cystic fibrosis transmembrane conductance regulator) regulates the insertion and removal of membrane on the surface of pancreatic cells - one of the cell types affected by CF. A defect in this process could potentially cause a host of changes in both the cells and their environment, since membrane insertion and removal is an important way for cells to control both secretion and placement of proteins on their surface. When membrane-bound vesicles inside the cell fuse with the plasma membrane that surrounds the cell, proteins inside the vesicles are released to the outside, and proteins in the vesicle membrane become part of the plasma membrane.

The inspiration for the group's experiments came from the finding of their Alabama colleagues Eric Sorscher and Ray Frizzell that a CFTR-containing cell line adds membrane to its surface in response to the intracellular messenger, cyclic AMP (cAMP). Since cAMP triggers CFTR-related chloride conductance, Kirk and Bridges wondered whether CFTR might also play a role in cAMP-stimulated membrane insertion, or exocytosis. To find out, the team studied a line of pancreatic cells derived from a tumour in a CF patient. The cells contain defective CFTR, and the researchers found that they do not have cAMP-stimulated exocytosis. But when normal CFTR was added to the cells, cAMP did trigger exocytosis, and also inhibited endocytosis, or membrane removal. This suggested CFTR was controlling both processes.

"I think it is quite an important observation, that CFTR is not a protein that just does its job in the plasma membrane but is also involved in intracellular processes", says Michigan's Collins. "It will open a new area of investigation."

Despite such enthusiasm on the part of some of his colleagues, Kirk is quick to warn against making too much of the finding. "The most I would want to interpret from our work is that CFTR is a participant in the regulation of exocytosis and endocytosis", he says. By doing so, it could be influencing the placement of important proteins on the cell surface, but there is no

evidence yet that that is so. And, he adds, several other big issues remain to be resolved: does CTFR play a similar role in other cells known to be affected by cystic fibrosis? If it does, how does it control the membrane flow from the surface of the cell, or from the membrane-bound compartments inside the cell? And is it acting as a chloride channel, or in some completely novel way? (Extracted from *Science*, Vol. 256, 24 April 1992)

New vector puts payload on the outside

The quest for the perfect gene delivery system has at times seemed one of those impossible dreams. In the eight years since scientists have been packaging healthy genes in viruses for delivery into cells, they have tested a dazzling and imaginative array of potential viral vectors in search of the one that combines maximum efficiency with optimum safety - but all approaches fell short of perfection, because those that were efficient were not always safe. One reason for the problems was that in every one of those approaches, the DNA was packaged inside the virus, and this meant viral genes were transported into the cell along with the therapeutic genes. A team of scientists from the University of North Carolina at Chapel Hill and the Research Institute of Molecular Pathology in Vienna, Austria, have announced a new vector that takes advantage of a virus's ability to get inside of cells, while inactivating the virus's genes. Instead of packaging the DNA inside the virus, they hook it onto the outside of the viral shell.

Reporting in the April issue of *Human Gene Therapy*, David Curiel, Ed Hu and colleagues at the University of North Carolina at Chapel Hill and collaborators Ernst Wagner, Matt Cotten and Max Birnstiel at the Research Institute of Molecular Pathology in Vienna, Austria, describe the new vector, which in addition to its potential safety advantages can carry more DNA than traditional viral vectors.

As innovative as it is, however, this "new" vector is but a technical variation - though an intriguing one - on a well-established theme. Researchers in laboratories interested in curing respiratory illnesses like cystic fibrosis have used the same virus - adenovirus - because it is able to target the epithelial cells lining the respiratory tract. But until now, adenovirus had a large drawback - it carries with it its own genes. As Curiel puts it: "The good thing about adenovirus is that it enters cells, and that's what we want. The bad part is that it carries with it its own genes."

The capacity for entering a cell, Curiel notes, resides in the virus's outer protein shell; none of the viral genes are needed for entry or contribute to the system's efficacy. So Curiel and his colleagues have either deleted or otherwise inactivated the adenoviral genes. But the real innovation lies in their decision to tether the DNA to the outside of the viral coat using a chemical linker.

The transporter consisting of the virus and its linked DNA enters the cell via a surface receptor and gets taken into the nucleus via normal cellular uptake and transport processes. Once in the nucleus, the therapeutic gene can be expressed along with native host genes. In test tube studies, the imported genes were expressed at a high level, rivalling the best of the traditional gene delivery systems. Since developing the system, Curiel and his colleagues have been stunned by its potential versatility. Part of that versatility comes from the linker that attaches the DNA to the viral coat. The linker binds to the virus by means of an antibody molecule specific for adenovirus. At the other end of the antibody is a chain made up of lysine amino acid units. Lysine, notes Curiel, combines indiscriminately and spontaneously with nucleic acids, so just about any nucleic acid - not just DNA but also RNA - can be fastened to the virus. This, he says, may make the system useful for antiviral therapies that require antisense RNA as wel' as for gene therapy.

And there are other benefits to putting the DNA on the outside. Until now, the size of the viral shell, like a suitcase stuffed to bursting, dictated the size of the DNA molecule that could be transported, with most viruses capable of transporting molecules no larger than 7,000 bases - slightly more than the cystic fibrosis gene without its regulatory regions. In contrast, Curiel and his colleagues have used the new vector to transport 48,000 bases of DNA successfully. They are now gearing up to test their system with yeast artificial chromosomes that include several hundred thousand bases. (Source: *Science*, Vol. 256, 24 April 1992)

T-cells involved in rheumatoid arthritis discovered

The Immune Response Corp. has discovered specific T-cells in the joint tissue of rheumatoid arthritis patients that are implicated in the destruction of joints in these patients. Preventing the attack by these cells may halt the rheumatoid arthritis disease process and provide an effective treatment for the millions afflicted with this disease.

The company has discovered three closely related populations of T-cells, VB17, VB14 and VB3, in the joints of rheumatoid arthritis patients. Immune Response believes that these T-cells initiate the autoimmune attack against the joint tissue in rheumatoid arthritis patients. The T-cells then recruit other immune cells to the joint, initiating a cascade of events that leads to inflammation, pain and joint destruction.

Traditional therapies for rheumatoid arthritis have addressed the disease symptoms, but have not halted the underlying attack against joints by the immune system. Immune suppressive therapies may inhibit this attack, but also weaken the immune system's ability to fight infections and disease. With the discovery of specific T-cells causing rheumatoid arthritis, therapies can now be

targeted at this small population of T-cells without compromising the immune system.

Immune Response is developing therapeutic vaccines based on receptors found on these specific T-cells. The vaccines are intended to stimulate the immune system to inhibit or destroy these disease-causing cells and thereby prevent the destructive immune cascade at its initiation. Such an approach could provide a long-lasting, safe and effective treatment for rheumatoid arthritis. (Source: *BFE*, Vol. 9, No. 1-2, January/February 1992)

University gene beat

Protein markers may provide clue to eradicating rheumatic fever

University of Florida (Gainesville) researchers have discovered special protein markers in the cells of people with rheumatic fever that could lead to the prevention of the disease. They have found that a protein called 70-KD occurs in high concentrations only in the cells of patients with rheumatic fever or the parents of those individuals. This suggests a possible genetic predisposition for contracting the disease, explained Dr. Elia Ayoub, chief of paediatric infectious diseases at UF's College of Medicine.

"The presence of the protein markers appears to be related to rheumatic fever and the susceptibility of an individual to develop it. Further research using the protein also may reveal the reason why certain people who become infected with strep develop rheumatic fever and why others do not", Dr. Ayoub said. Since these markers may give physicians the ability to identify individuals who are likely to develop rheumatic fever, people with the protein present could be placed on antibiotic therapy to prevent the development of the disease.

Scientists discover tumour antigens

Dr. Thierry Boon and co-workers at the Ludwig Institute for Cancer Research in Brussels have found three related human tumour antigens on human melanoma cells. The proteins provoked strong killer T-cell activity in test tube experiments, and were found on the cancer cells, but not on normal cells of the skin, lung, liver and elsewhere in the body.

These findings suggest that the antigens may be mutations that occur as part of the transformation of normal cells to cancerous ones. In addition, they may provide good targets for a vaccine.

Purdue researchers decipher code of virus protein

New findings at Purdue University (W. Lafayette, IN) provide insights on the role played by proteins in the life cycle of a virus, and furnish a detailed picture of the shell of a lipid-coated virus.

"Generally, a protein has a single function in a virus", says Michael G. Rossmann, Purdue's Hanley Distinguished Professor of Biological Science. "In this case, the viral protein shell performs a number of tasks, acting almost as a nerve centre for the virus."

The team mapped the Sindbis virus, which is carried by insects and can cause various complications in humans, including encephalitis, fever, arthritis and rash. The three-dimensional map they developed, in collaboration with a team at the University of Giessen in Germany, reveals that the protein shell of the virus has a distinct structure that allows it to play many roles in the life cycle of the virus. One of the main functions of this protein is to act as a serine proteinase. Its structure is similar to certain digestive enzymes, and may help scientists understand how other proteinases perform their functions.

Duke researchers reveal structure of brain protein

Researchers at Duke University Medical Center (Durham, NC) and Emory University (Atlanta, GA) have isolated and cloned the gene for a brain protein believed to play a role in depression and other psychiatric syndromes. The protein is a transporter that carries serotonin between nerve cells. Scientists believe that some emotional behaviours are physio-logically controlled by the movement of certain neurotransmitters between brain synapses. Serotonin, found in the mid-brain and brain stem, is known to regulate affective behaviour. Researchers know that patients with depression, obsessive-compulsive disorders, panic disorders, bulimia and obesity, show abnormalities in the transmission of serotonin. By knowing the structure of the transporter proteins, and isolating and cloning their genes, researchers can design drugs that will fit into the transporter, thereby limiting the uptake of serotonin, and reducing the affective disorder that the serotonin causes.

Jefferson researchers identify gene that binds skin

Jouni Utto, M.D., Ph.D., and co-workers at Thomas Jefferson University (Philadelphia, PA) have identified a collagen gene responsible for holding skin together and have linked a site on this gene, found in several large families, to a form of epidermolysis bullosa (EB), a severe, inherited skin disease. In separate studies using normal cultured skin cells, researchers were also able to stimulate the gene to increase collagen production nearly seven-fold.

The findings confirm long-held assumptions about the role of type VII collagen, a major component of anchoring fibrils found in normal skin where the epidermis and dermis meet. These fibrils bind the inner

and outer layers of skin together and play a role in wound healing and in keeping skin taut.

The genetic finding provides a means of identifying carriers of the "bad" gene among families with dominant dystrophic epidermolysis bullosa, a form of EB that causes skin to form blisters within the dermis and peel in response to minor contact. Based on preliminary findings in cell cultures, researchers hope to develop topical ointments, autologous skin grafts and other skin-repairing treatments for this disease, by stimulating the gene to produce healthy protein fibrils. (Extracted from *Genetic Engineering News*, January 1992)

Gene for vital enzyme cloned

Researchers in Japan report the first determination of the genomic structure and chromosomal location of the human gene for aromatic L-amino acid decarboxylase (AADC). The findings are important because AADC catalyses the conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxy-tryptophan (L-5HTP) to dopamine and serotonin, respectively, key neurotransmitters in the central nervous system. The genetic data could also be useful for analysing the molecular mechanism behind recently reported cases of AADC deficiency. The study was conducted by Toshiharu Nagatsu of Fujita Health University (in Aichi) and co-workers there, at Nagoya University School of Medicine, and at the National Institute of Radiological Sciences (in Chiba). In addition to cloning the gene and assigning it to chromosome 7, the researchers addressed the question of whether decarboxylation of L-DOPA and L-5HTP are mediated by the same enzyme, AADC, in view of differences in kinetic parameters of the two reactions and other evidence. They find AADC catalyses both decarboxylations, but that minor changes, such as from post-translational modifications, could account for the disparities found in previous studies. (Reprinted with permission from *Chemical and Engineering News*, 16 March 1992, p. 26. Copyright (1992) American Chemical Society)

Research on animal genes

Mitsubishi Kasei isolates cartilage growth factor

Mitsubishi Kasei Corp., Tokyo have announced that joint research with Fujio Suzuki of Osaka University has led to isolation of a cartilage cell growth factor from foetal bovine epiphysial cartilage. The factor is a protein with a molecular weight of about 25,000 and consists of 121 amino acid residues. According to Mitsubishi Kasei, the factor shows potential for the treatment of bone fractures and disorders of bone and cartilage. The company plans to continue with animal experiments to further clarify structural and functional aspects of the compound. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 January 1992)

Ancient bees buzz back to life

Quirks of preservation sometimes offer an extraordinary glimpse of the past, as a team of molecular biologists is learning. The American researchers have shown that stingless bees entombed in sticky resin between 25 and 40 million years ago contain surprisingly intact fragments of DNA.

The bees, found in the Dominican Republic, now hold the record for the oldest DNA. As impressive as the age of the bee DNA is, the size of the fragments is even more important. Most ancient DNA is found in strings of about 500 nucleotide bases; 1,200 bases is the record. The bee DNA is reportedly between 6,000 and 10,000 bases long. That should be enough to provide a snapshot of the ancient past. (Source: *New Scientist*, 4 April 1992)

Cancer compound found in cuttlefish

A mysterious ingredient in cuttlefish ink may be effective in fighting cancer, say researchers in Japan.

Jin'ichi Sasaki and his colleagues at Hirosaki University in northern Japan partially purified cuttlefish ink into a mixture consisting mainly of a conjugated glucide (in which sugar, protein and lipid units are combined). They tested it on 15 mice which they had implanted with tumours.

The researchers injected each mouse with three 200-milligram doses of the compound on the second, fourth and sixth days after the tumour transplants. They found that nine of the mice recovered and were still healthy two months after the experiment began. However, a control group of 15 mice, which were given no injections, all died within three weeks.

Sasaki's group, and another team working from the nearby Aomori Advanced Industrial Technology Centre, are now planning to identify and isolate the active ingredients in the ink, and then synthesize it. Although the study is in its early stages, Sasaki says he is encouraged by the fact that 60 per cent of the mice that they tested recovered.

He believes the compound works by activating macrophages, a type of white blood cell, near the site of the tumour. This would increase the body's immune response to the tumour cells rather than fighting the cancer cells directly.

Sasaki recently published the work in the Japanese journal *Biotherapy*. (Source: *New Scientist*, 25 April 1992)

Pesticide based on spider toxin gene

The significance of spiders to farming was strengthened last week when scientists at Salt Lake City-based Natural Product Sciences Inc. (NPS) and FMC Corporation announced that NPS has successfully produced in its laboratories a genetically-engineered, orally-active pesticide based on a spider toxin gene.

The work is the result of a three-year collaboration between NPS and FMC. Both companies believe that biopesticides of this type represent a new, environmentally-safe approach to crop pest control, a multi-billion dollar per year industry.

NPS scientists have identified a spider toxin that is lethal to major crop pests but apparently harmless to mammals, including humans. This toxin breaks down so rapidly in the environment, it cannot be used like a conventional chemical pesticide.

In nature, the toxin is delivered to the insect as part of the venom the spider injects into its prey. In fact, the toxin itself is not effective when touched or eaten. While this provides an even greater level of environmental safety it creates a technical problem for the practical delivery of the molecule.

To deliver this fragile molecule to its insect targets, NPS has inserted the cloned spider toxin gene into a virus exclusively infectious to certain insects. The virus begins producing spider toxin internally when the insect eats the virus, leading to paralysis and death of the insect.

NPS has also removed a different gene from the virus to add another layer of environmental safety. The removed gene would normally allow the virus to survive in the soil after the insect host dies. (Source: *Chemical Marketing Reporter*, 20 January 1992)

Of mice and men ... and rabbits

Ben Koop of the California Institute of Technology reported comparisons of long stretches of DNA between humans, mice and rabbits, including one that was a staggering 96,000 bases long. This is the most extensive comparison of DNA between species, and reveals something of the Byzantine ways in which DNA may alter through time.

In their first comparison, Koop and his colleagues sequenced a region of DNA in humans and mice that codes for certain molecules that interact with T-cells. This section of DNA (the 96,000 bases) contains regions that specify genes and other regions that probably code for nothing and are known as spacers. Koop and his colleagues compared the 96,000-base DNA strings from humans and mice, and found two surprises.

First, although humans and mice have been evolving separately for some 65 to 85 million years, between 67 and 70 per cent of the DNA sequence was the same in the two species. Moreover, the gene coding and spacer regions had accumulated about the same number of mutations, suggesting that mutation is kept in check for the whole region, not just the sections coding for genes.

Second, the order of the various gene-coding and spacer regions along the 96,000-base DNA string is very similar in the two species.

To address this question, Koop and his colleagues included the rabbit in their comparisons, and also analysed a region of DNA known as the beta globin region, which codes for proteins in red blood cells. As with the T-cell region in humans and mice, there was a 67 per cent similarity in the beta globin regions of humans and rabbits - and little difference in overall organization. However, there was a tremendous difference in the way genes are organized in the human and mouse beta globin region.

Clearly, different things were going on in the two gene regions. "Many more gene regions will have to be examined before we can say which of the three is the most typical", said Koop. (Source: *New Scientist*, 22 February 1992)

Life-span doubled in worms, flies, but Fountain of Youth still eludes humans

For *Drosophila* - fruit flies - 40 days is ripe old age. But a combination of anti-ageing methods had 40-day-old *Drosophila* buzzing around like teenagers. Methods used to delay ageing in the flies included dietary restriction and "tuning" natural selection by encouraging the reproduction of flies with a built-in stress resistance and an ability to reproduce late in life. In many species, deterioration begins once an organism reaches the reproductive stage. Evolutionary biologist Michael R. Rose has managed to breed fruit flies that survive for the human equivalent of 150 years.

With mutation of a single gene, senescence in the soil-dwelling nematode - *Caenorhabditis elegans* - has been delayed by 70 per cent. In *C. elegans*, the dramatic increases were caused by a mutation in one gene, named age-1, that decreased the worm's fertility, but boosted longevity. By chemically mutating the gene, Thomas E. Johnson of the Institute for Behavioral Genetics, University of Colorado, achieved a twofold extension of the worm's three-week life-span. His group is trying to clone the age-1 gene, in hopes of conducting a gene therapy experiment that will increase life-span.

Johnson intends to look for similar genes in mammals, in hopes of increasing life-span through genetic manipulation, he said. The ultimate goal is a

human life of 130 years. In the future, the scientists predict that anti-ageing gene therapy may be possible, although they are reluctant to speculate about the exact nature of such a procedure. One major drawback, they agreed, is that it will most likely take several genes to delay ageing in higher animals. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Nichirei produces glycoproteins in animal cells

Researchers at Nichirei Corporation, Tokyo, in collaboration with Professor Koichiro Oda of the Science University of Tokyo, have developed cost-efficient technology to produce glycoproteins in transformed animal cells. Physiologically active proteins can be produced in recombinant *E. coli* cells, but the microbes are unable to biosynthesize glycoproteins. Conventional production of glycoproteins in transformed animal cells needs costly serum media and yields small quantities of the desired products.

The starting point for the joint team's research was the observation that the fibronectin promoter gene, pF1900, is highly active during cell interphase. The team produced a construct containing this promoter linked to a target gene and inserted it into the rat cell line, 3Y1, suspended at the Go stage of interphase. The team then cultured the cells in an inexpensive serum-free medium. According to the results of experiments using the fibronectin promoter linked to the gamma-interferon gene, the rat cells produced the glycoprotein with an efficiency equivalent to that of *E. coli*. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Modification of mammalian development accomplished by gene manipulation

Researchers at the Institut Pasteur (Unité de génétique cellulaire) have succeeded in modifying the developmental process of mouse embryos, by engineering the Homeobox (Hox) genes. This family of Hox genes determines the sequential development of structures in animals, for example, limbs or the backbone in verte-brates or segmentation in flies. Using the technique of homologous recombination they have succeeded in overcoming the limitations of the random gene insertion, which has characterized transgenic animal research to date.

Philippe Brûlet, Hervé Le Mouellic and Yvan Lallemand took embryonic stem (ES) cells, that is undifferentiated or totipotent cells from very early mouse embryos, and the Pasteur team was able to replace the Hox-3 gene with a non-effective marker gene. Affected mice grew up with anomalies of the vertebra. In effect vertebrae at the rear of the animal had the morphological characters of those at the front.

This remarkable piece of gene engineering necessitated the grafting of the marker gene in the right copy number and in exactly the right position with regard to all control elements. This was achieved through the technique of homologous recombination. The success of this experiment marks an important first for the Pasteur and for the medical applications of gene manipulation. Gene therapy now becomes achievable, as Hervé Le Mouellic says, by changing not the position but the identify of cells which express Hox genes.

For all concerned with the developmental process this publication marks one of the most important events of the decade, giving for the first time direct access to the processes of developmental control. Obvious first applications involve the development of animal models for a number of genetically determined developmental malfunctions. (Source: *BFE*, Vol. 9, No. 6, June 1992)

Rats cured of Parkinson-like symptoms

Transplanting genetically modified cells can reduce symptoms similar to those of Parkinson's disease. A group of doctors in California has used the cells in rats, and says the animals have shown a marked improvement in their behaviour and movements. The researchers believe that cells genetically manipulated in this way have great potential for treating diseases of the central nervous system such as Parkinson's disease in humans.

In their experiments, Rein Anton at the University of California in Los Angeles and his colleagues used brain cells of a type called nigral cells which produce the neurotransmitter dopamine. The researchers inserted a gene which allowed the cells to proliferate indefinitely at a temperature below body temperature into the cells. In this state, the cells are said to be "conditionally immortalized".

Rats in which symptoms of Parkinson's disease had been experimentally induced were given transplants of these brain cells. It was found that the transplanted cells produced a 70 per cent reduction in the rats' symptoms. When the genetically modified cells are implanted, they reach body temperature, stop growing, and send out sprouts. But they still produce dopamine, says Anton. The technique does not require the use of human foetal tissue, and all the cells used are descended from a single brain cell. According to Anton, the conditionally immortalized cells can be made available in unlimited quantites, and can be genetically manipulated at below body temperature. Yet they remain capable of performing their specialized role at body temperature.

Anton believes there is considerable potential to improve his cells through further genetic engineering. (Source: *New Scientist*, 20 June 1992)

Research on plant genes

New gene-transfer technology

Plant Genetic Systems (Belgium) has succeeded in getting corn to keep from self-pollinating, thus opening the way to stronger and economically important hybrid plants. The firm used a new gene-transfer technology to make corn plants male-sterile without having to manually or mechanically remove the male part (i.e., the tassel) of the corn plant. According to Herman Van Mellaert, business development manager at PGS, detasseling costs the US corn-seed industry about $200 million per year. It may take three years for the new hybrid seeds to reach farmers, according to Ronald Holden, president of Holden's Foundation Seeds (Williamsburg, IA). The company provided its corn seeds to PGS to be impregnated with the new genetic material. PGS said the significance of its work with corn is that its method of transferring genetic material is more efficient than other techniques currently in use. (Extracted from *Wall Street Journal*, 15 April 1992)

MAFF okays open-air transgenic tomato trial

Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) has announced approval of an application from the National Institute of Agro-Environmental Science (NIAES) for open-air cultivation of a transgenic tomato breed. NIAES researchers genetically engineered tomato plants to resist the tobacco mosaic virus (TMV), a serious pathogen of the species. The researchers tested the breed through a series of controlled cultivation tests run in collaboration with groups at the National Institute of Agrobiological Resources (NIAR) and the National Agriculture Research Center (NARC). Having won approval to cultivate the transgenic breed, the NIAES group will use it to develop commercial TMV-resistant breeding stock. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Genetic weeding and feeding for tobacco plants

Biochemists in Germany have found a way of protecting tobacco crops from weeds, while simultaneously providing them with fertilizing nitrogen. They have genetically engineered the tobacco plants to produce an enzyme to convert the common weed killer cyanamide (H_2NCN) into urea (H_2NCONH_2). Not only are the plants then resistant to the herbicide, but their natural enzyme can further convert urea into useful nitrogen compounds.

A 5 per cent solution of cyanamide kills the weeds which infest tobacco plants (*Nicotiana tabacum*), but causes some damage to the tobacco as well. In the past, researchers have enhanced the plant's resistance to herbicides by introducing genes which encode enzymes which degrade the herbicides. But this has disadvantages. The breakdown products have often proved to be physiologically active and capable of damaging the plant.

Guido Hartmann and his colleagues at the Ludwig Maximilian University in Munich have found a way around this problem by ensuring that the breakdown products are useful to the plant. They have engineered resistance into tobacco plants by inserting a gene from the soil fungus, *Myrothecium verrucaria*. The gene codes for the enzyme cyanamide hydratase, which converts cyanamide to urea by catalysing the addition of a water molecule. The researchers believe that it might be possible to extend the technique to other crop plants. (Source: *New Scientist*, 4 January 1992)

Iron-removing algae found in reconstructed prairie

Biologist Wayne Nichols of Washington University, St. Louis, has found a new species of algae, *Sphaerellocystis aplanosporum*, that removes iron from the soil. He isolated the species, and more than 500 others, from the soils of a restored prairie near Springfield, Missouri. The restoration is part of a move of the past 20 years to re-create prairie of the kind that covered the Midwest before about 1848, when the sodbusters, equipped with John Deere's steel plough, began converting the prairie to farmland.

Nichols is interested in the dynamics of the prairie ecosystem. The iron-removing alga, if left on its own, for example, would deplete the soil of that essential metal. Other organisms, however, create a system of checks and balances that seems to keep everything working well. Nichols and his colleagues think algae play a more complex role in soil than people have believed. They hope to develop a biological profile of prairie algae that may serve as a barometer of change in the prairie environment. (Abstracted with permission from *Chemical and Engineering News*, 9 December 1991, p. 76. Copyright (1991) American Chemical Society)

Patented soybeans convert more nitrogen

Agrigenetics Co. has been granted a United States patent for soybeans with a unique gene that increases their nitrogen-fixing capacity. Compared to ordinary soybean plants, the supernodulating variety grows roots with more of the bacteria-producing nodules that turn atmospheric nitrogen into nitrogen fertilizer. Economic benefits can include higher soybean yields, lower commercial fertilizer requirements and organically improved fields for crop rotation.

Peter Gresshoff developed the genetically engineered soybean at the Australian National University under a research grant funded by Agrigenetics, which continues to study the supernodulating trait in its own laboratories.

Product planning manager Robert B. Ratliff said "The ultimate goal is to teach plants like corn that don't fix their own nitrogen to do so in the future." According to Ratliff, the company expects to make available demonstration quantities of the product in 1993 and marketable amounts by 1994. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 June 1992)

Modified wheat paves the way to bumper harvest

Genetically engineered wheat has been produced for the first time. This breakthrough paves the way for improved versions that resist drought, disease and weeds, which together reduce harvests by a quarter or more.

A team at the University of Florida at Gainesville, led by Indra Vasil, introduced a gene into wheat that makes it resistant to a herbicide called *Basta*. This trait was passed on to successor generations, grown from the seeds of the first line, showing that the new gene is inheritable.

The researchers took the gamble of injecting foreign DNA directly into the callus. "We had little hope that this would work, yet it did", says Vasil. The cells acquired resistance to *Basta*.

Only four pieces of callus out of 640 had accepted the new gene, but this proved to be enough. Vasil went on to grow 100 transformed plants, 75 of which survived in soil and grew to maturity. He says that they have now grown three generations of plants from the original line, and these show complete resistance to Basta.

Vasil believes his breakthrough could be vital to feeding the world's rapidly growing population. He hopes to use similar methods to produce strains resistant to viruses and fungi, or which yield grain containing more protein.

Vasil wants to provide poorer countries with cheap access to the technology, and says that negotiations to this end are under way at UNESCO. He says developing countries are "where the maximum need and impact is going to be".

The University holds a patent on the technology, but has granted an exclusive licence to the chemical company Monsanto. The company collaborated with Vasil in the research and provided the team with the gene conferring resistance to *Basta*. (Source: *New Scientist*, 27 June 1992)

Research on yeast and fungus genes

Two-year study leads pan-European team to major breakthrough

Humble yeast, a vital ingredient in industries from brewing and baking to pharmaceuticals, is at the centre of a breakthrough in European microbiology.

For the first time, scientists have analysed the chromosome of a living organism which they believe will prove a vital step towards understanding the complex genetic make-up of plants, animals and man. Research into *Saccharomyces cerevisiae* - plain bakers' yeast - is claimed to be the perfect training ground for later study into more complex organisms.

The pan-European study was itself unique - a collaboration between 147 researchers in 35 laboratories and funded by the European Community.

The results of the study, published in the scientific magazine Nature, followed two years' work. The yeast organism is small and therefore easy to handle in the laboratory, but, unlike bacteria, it is a more complex organism, many features of which control the same basic functions as those in higher organisms, including man.

More than 4,000 scientists world wide are involved in research to map the yeast genome, the organism's unique biological make-up.

The European research project, which received Ecu 2.4 million from the EC's Biotechnology Action Programme, was coordinated by Professor André Goffeau of the Catholic University of Louvain.

The scientists have isolated chromosome III, one of yeast's 16 chromosomes, and work is under way to analyse a further three. Laboratories in the United States, Canada and Japan are also working on a further three chromosomes. By the year 2000, it is hoped the yeast genome will have been sequenced, with more than half the work undertaken by European laboratories.

Scientists are now working to discover the exact function of the chromosome they have isolated, but there are no plans to patent it until this is known. Patenting has been hotly disputed in the United States, where scientists have rushed to patent fragments of genomes without being aware of their function. Some fear that patenting of gene fragments will stifle research, while others claim that researchers must reap the financial rewards of their work if the gene's function is important.

The possibilities for a biotechnology-engineered yeast are enormous. It could cut costs and improve efficiency in a range of agri-foods industries and offers the possibility of new medicines and vaccines. Yeast is already an ingredient in the manufacture of a vaccine for hepatitis B and the drug Interferon.

Ironically, one of the obstacles for the European Yeast Genome Sequencing Consortium was communication. Many laboratories claimed not to know of each other's existence but are now working together and for the first time believe there is a "yeast grapevine"

that will speed future work. (Source: *The European*, 7-10 May 1992)

Yeast gives semiconductors a lift

The tiny semiconductor structures that will let future microchips compute with light rather than electricity may be manufactured by yeast. Researchers from the Advanced Centre for Biochemical Engineering at University College London (UCL) told a recent conference in Manchester that they have succeeded in making tiny semiconductor structures from cultures of a yeast called *Schizosaccharomyces pombe*.

The yeast produces structures known as quantum semiconductors, bits so small that the electronic properties of the material are affected by the close proximity of the edges. Researchers can tailor the size and shape of such structures to give them properties that bigger pieces of the same material do not have.

Quantum semiconductors can be made to behave like lasers and emit light, or shaped into channels for light known as waveguides. Light travels much faster than electrical signals, so such structures will be important in future optical computers that use light rather than electrons. The problem, however, is that the structures need to be so small that conventional manufacturing techniques are often stretched to their limits.

Paul Williams and Eli Keshavarz-Moore of UCL make the structures by adding cadmium sulphate to a yeast culture. The yeast protects itself against the toxic cadmium by coating the compound with a shield of peptides. In the process, the yeast converts the compound into tiny particles of cadmium sulphide, which is a semiconductor.

Importantly, says Williams, the particles of cadmium sulphide produced - each about 1·8 nanometres in diameter - are very uniform in size, much more so than if they are produced by chemical or synthetic means. This means that the electrical properties are more predictable.

The researchers tested their properties by illuminating them with ultraviolet light. The particles luminesced with a spectrum similar to that from cadmium sulphide particles produced by other methods. Bulk cadmium sulphide does not luminesce in this way. (Source: *New Scientist*, 25 January 1992)

Research on bacterial genes

Nitric oxide damages DNA in bacteria

Researchers have found that nitric oxide (NO), a bioregulatory agent in the human body and an environmental pollutant, can damage DNA in living cells. According to chemist Larry K. Keefer of the National Cancer Institute's Frederick (Md) Cancer Research & Development Center and his co-workers, nitric oxide can deaminate cytosine and other DNA bases. The reaction sequence, which proceeds at physiological pH, involves nitrosation and diazotization of the base's exocyclic amino group, followed by hydrolysis of the resulting diazonium ion. Converting the amino group to hydroxyl in this way leads to a base change, and thus to a heritable genetic alteration. This was demonstrated by exposing the bacterium used in the well-known Ames mutagenicity assay to three nitric oxide-releasing compounds, including the therapeutic agent nitroglycerin. All three were found to be mutagenic, the specific mutation being cytosine to thymine. This mutation is consistent with a cytosine deamination mechanism, the researchers say. The same mutation in humans has been linked to a variety of disorders, including haemophilia, familial Alzheimer's disease, and colon cancer. Thus, they conclude, nitric oxide produced metabolically and in cigarettes, for example, may contribute to the incidence of genetic disease and cancer. (Reprinted with permission from *Chemical and Engineering News*, 18 November 1991, p. 23. Copyright (1991) American Chemical Society)

Bizarre bacterium in a class of its own

An unusual bacterium has turned up in a dam in Queensland, Australia. Its structure is more like that of a plant or animal cell than any other bacterium. The bacterium does not fit into the traditional classification scheme of living organisms, and promises to give biologists new insights into how life evolved.

In all other bacteria, genetic material floats freely within the cell. But in the Australian oddity, which has been named *Gemmata obscuriglobus*, DNA is packed within a nuclear membrane. This is what makes this bacterium special, says John Fuerst of the University of Queensland, the leader of the team investigating it.

The presence or absence of a nucleus was the observation which led scientists to divide living organisms into two groups: "prokaryotes", which do not have a nucleus; and "eurkaryotes", which do. Only bacteria are prokaryotes; all other living things are eukaryotes.

Gemmata is a problem, says Fuerst, because it does not fit neatly among either prokaryotes or eukaryotes. It is a bacterium in all respects, except that it has a nucleus.

The peculiar freshwater bacterium was first isolated in 1984 by Peter Franzmann of the University of Queensland. Guy Cox of the University of Sydney has collected a similar organism from cave walls in New South Wales, but he has not yet proved that the cell structure is the same. Fuerst and Richard Webb, also of the University of Queensland, began electron microscopic investigation of *G. obscuriglobus* in 1987.

The scientists at the University of Queensland are trying to determine whether the bacterium is ancient or a later arrival. (Source: *New Scientist*, 25 January 1992)

Bacterial image detector spots anything that moves

A humble marine bacterium may be the key to robot vision systems which have hitherto existed only in the realms of science fiction. Using a protein derived from the purple pigment of *Halobacterium halobium*, Japanese researchers have taken the first steps by making a basic image detector with characteristics similar to those of a real retina.

The protein, called bacteriorhodopsin, is similar to the protein rhodopsin, which detects light in a real retina. In the middle of the bacteriorhodopsin molecule is a non-protein component called retinal. The energy of a photon causes the retinal to change shape, from straight to bent. Some atoms in the retinal carry a charge, and when it changes shape the charges move with respect to each other. This movement creates a potential difference in the retinal, which can be used to generate an electric current.

To build their detector, Tsutomu Miyasaka and colleagues from the Fuji film company in Kanagawa, Japan, created a film of bacteriorhodopsin just one molecule thick on the surface of water, and transferred it onto a plate of glass coated with a transparent layer of tin oxide. The oxide is an electrical conductor, so acts as an electrode.

Bacteriorhodopsin does not react to steady incoming light but to changes in intensity. The Japanese photocell therefore produces a pulse of current when first illuminated which drops to nothing if the light stays constant. When illumination is turned off, the protein produces a pulse of current in the opposite direction. This feature of the photocell is what makes it most like a real eye. Rhodopsin in a retina behaves the same way, and this helps animals and humans to spot moving objects against an unchanging background.

The Japanese researchers have gone on to make another photocell where the tin oxide electrode has been replaced with a square array of 64 electrodes made of indium-tin oxide printed on the glass. Each square in the array is 2.5 millimetres across and is connected to a light-emitting diode in a matching array of 64 LEDs. Each time the bacteriorhodopsin sends a current pulse through one of the electrodes, its corresponding LED lights up.

The researchers shone images of letters at the photocell array. Nothing appears on the LEDs when the letters are shone steadily at the screen. However, when the letters move or are flashed on and off very quickly, their image appeared on the array of LEDs. (Source: *New Scientist*, 25 January 1992)

"Molecular thermometer" found in common bacteria

The mystery of how cells detect changes in temperature may have been solved by two American biologists. John McCarty and Graham Walker at the Massachusetts Institute of Technology in Cambridge, Massachusetts, have found a protein in the bacterium *Escherichia coli* which detects changes in temperature. It also controls the organism's response to the changes. The researchers think that similar protein "thermometers" may be used by plants and animals.

McCarty and Walker made their discovery while studying the "heat shock response" in cells. When a cell's temperature changes, it rapidly makes a set of proteins known as the "heat shock proteins" (HSPs). These "molecular chaperons" bind to other proteins, helping them to fold into the correct shape, for instance. Proteins are less stable at higher temperatures, so the cells may be producing HSPs to compensate for this.

HSPs are found in all living organisms, from arctic fish to tropical plants. Because they are similar in widely different organisms, biologists believe they must have arisen very early in evolution.

The heat shock protein that McCarty and Walker studied, HSP70, not only acts as a molecular chaperon, it also forms part of the network of proteins which controls the heat shock response itself.

Biochemists have studied HSP70 before. When it binds to a protein, it can release the protein only by breaking down adenosine triphosphate (ATP), the molecule which drives chemical reactions in cells. Also HSP70 comes in two forms: one is "phosphorylated", with a phosphate group attached to it, while the other form is not.

McCarty and Walker found that only the phosphorylated form of HSP70 can break down ATP in *E. coli*. This implies that only this form is able to detach itself from a protein which is chaperoning it.

As part of their study, McCarty and Walker gradually increased HSP70's temperature from 20° C to 50° C. As the temperature rose, more and more HSP70 molecules converted themselves into the phosphorylated form. The relationship was regular: the percentage of phosphorylated HSP70 was a direct measure of temperature.

The American researchers suggest that because HSP70 is more likely to be phosphorylated at higher temperatures, it will release proteins more quickly and so chaperon more proteins. But HSP70 also binds to a protein called sigma-32, which activates the heat shock response. The researchers believe that as the temperature increases, HSP70 releases more sigma-32, freeing it to turn on the heat shock response.

All living cells contain HSP70, say the researchers, so plants and animals might also use the protein to detect temperature. One exciting possibility is that HSP70 may have a role beyond controlling the heat response. For instance, it may trigger impulses in temperature-sensitive nerve cells.

McCarty believes that if HSP70 has a more general role, other proteins may also be involved. Plant and animal cells, unlike *E. coli*, contain several different versions of HSP70. So in plants and animals this molecular thermometer may well have more than a single function. (Source: *New Scientist*, 11 January 1992)

Research on viral genes

Herpes virus turns tumour cells into sitting ducks

A form of gene therapy that was once dismissed as far-fetched should start trials in patients within months. The aim is to destroy tumour cells in the brain by introducing a gene from the herpes simplex virus into them. This makes the cells susceptible to the anti-herpes drug ganciclovir, which kills them.

So far, most gene therapy trials have involved introducing into cells a healthy copy of a gene that is faulty or absent. The new approach is to change the nature of the cell with a foreign gene, so that the cell can then be targeted with a specific drug. Like some other forms of gene therapy, the technique uses a mouse retrovirus to carry the gene into cells.

Kenneth Culver, who led a team at the National Cancer Institute in Bethesda, Maryland has approval from the National Institutes of Health to test the therapy in 20 people with inoperable brain tumours.

Scientists have been taken by surprise by the early success of the method in laboratory animals. But there are important safety hurdles to cross before the technique could be used more widely in humans. For example, retroviruses can insert their genetic material into the host's DNA, raising the risk of new cancers.

Culver used a mouse retrovirus, genetically altered to carry a gene from herpes simplex. The gene encodes an enzyme, thymidine kinase. When ganciclovir binds with this enzyme in cells, the cells die.

After initial experiments in mice, the team took rats with gliomas, a form of tumour which accounts for about half of all human brain tumours. They injected the tumours directly with mouse cells that were infected with the altered mouse retrovirus. These cells produced new virus particles, which went on to infect the glioma cells.

After five days, the team treated the rats with ganciclovir. Control animals, whose tumours had been injected either with saline or with uninfected mouse cells, were unaffected by the drug. By contrast, the tumours of 11 out of 14 animals that had received the retrovirus disappeared.

Retroviruses need to use their host's genetic material to replicate, and they can only make copies of themselves in cells that are dividing. In the brain, few healthy cells divide, and the retrovirus appears to have confined itself to cancer cells, which divide rapidly. Culver says there is no evidence of the virus spreading beyond these cells.

Even tumour cells untouched by the retrovirus were killed by the ganciclovir. This "bystander" effect has yet to be explained, says Culver. He is cautiously optimistic about the trials in humans. (Source: *New Scientist*, 20 June 1992)

Polio virus grows in cell-free broth

University at Stony Brook (Stony Brook, NY) scientists have synthesized infectious poliovirus in a test tube in a cell-free broth. It was previously thought that viruses could reproduce themselves only in whole, intact cells.

Microbiologists Akhteruzzaman Molla, Aniko Paul and Eckard Wimmer used an extract made from crushed human cells to grow the virus. They added messenger RNA for polio, as well as the raw materials for RNA and proteins, and the chemicals that supply the energy for making viruses.

Their discovery is particularly relevant to studies of viruses that cause diseases such as the common cold, meningitis, hepatitis and myocarditis, because these viruses are all related to poliovirus. (Source: *Genetic Engineering News*, January 1992)

Drug inhibits key HIV regulatory protein

A benzodiazepine designated Ro 5-3335 has been shown by Hoffmann-La Roche researchers to inhibit replication of the human immunodeficiency virus - the cause of AIDS - by acting as an antagonist of one of the virus's essential regulatory proteins. HIV's genome encodes a protein, designated Tat, that increases expression of the HIV provirus integrated into the genomes of cells infected with the virus. Tat functions by interacting with a sequence, designated TAR, in the proviral DNA. Hoffmann-La Roche scientist Ming-Chu Hsu and collaborators demonstrated that Ro 5-3335, which is 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepin-2(H)-one, blocks the interaction of Tat and TAR, leading to inhibition of HIV replication in an infected cell. Although Ro 5-3335 exhibits some toxicity in experimental animals, the compound is a useful model for analogs that might act by the same mechanism. Drugs that block HIV replication by inhibiting Tat could be attractive for use in combination with HIV reverse

transcriptase inhibitors such as zidovudine, which interfere with the process by which cells become infected with HIV. (Reprinted with permission from *Chemical and Engineering News*, 23 December 1991, p. 16. Copyright (1991) American Chemical Society)

Gene targeted pharmaceuticals approach

Currently, it is not possible to eliminate retroviruses such as HIV from infected hosts. Thus, the prospect of a "cure" for HIV infection is not promising. However, there is characteristically a period during which the impact of the virus is modest or negligible - it is considered to be "latent". Ultimately, with some retroviruses such as HIV, the health of the host begins a progressive decline. Thus, if the initial period of "latency" could be extended, patients would have a better chance to live out their normal lives.

The most promising way to recreate or maintain the latent state is to prevent the virus from activating its genes and making more virus particles. This contrasts to current drugs, which reduce the rate of infection of uninfected cells, but do not treat already infected cells.

Early work in the HIV field showed that the virus depends on a specific protein called "Tat" to turn on the expression of its genes - a process called "transactivation". Thus, inhibition of Tat function should maintain the virus in a latent state. Allelix Biopharmaceuticals therefore chose to develop inhibitors of Tat.

Allelix scientists focused on a particular step in Tat action - that of binding to a part of the viral genetic structure called TAR. They developed a series of molecules which effectively competed with Tat for binding to TAR in the "test tube". Two challenges remained: would these molecules get into cells and would they be stable enough that they could work before being degraded? By application of a number of techniques, a family of compounds were developed (ALX40-4) with these properties. One of these methods has led to a general approach to increase cell uptake of pharmaceuticals, which the company calls RG9, and which is the subject of recent patent applications.

Tests by Allelix and its collaborators, as well as by several major pharmaceuticals companies, have now demonstrated the capability of ALX40-4 to inhibit transactivation, and more importantly, HIV itself. These results manifest the considerable potential of the approach.

The company is now turning its technology to other targets to develop new and significant inhibitors of other human diseases. Lessons learned in the HIV project, such as RG9 technology, are very likely to help speed the development of these new compounds.

To date, pharmaceutical biotechnology has been based on technologies to manipulate genes in the "test tube". Recombinant methods to put new genes into cells so that they produce protein therapeutics such as PTH are the first practical applications of these technologies. But more recently, other opportunities have arisen. One emerging approach uses pharmaceuticals to alter the way genes are used within living organisms.

Allelix Biopharmaceuticals has been developing pharmaceuticals which regulate gene activity. In its first application, this approach has led to a novel class of inhibitors of human immunodeficiency virus (HIV) which have aroused considerable interest. (Source: *News Release*, 1991/92)

HIV enzyme finding sparks new programme

Recent discovery of a new enzymatic action in HIV promises to carry Bio-Technology General (BTG) into new research and drug development areas. The company's chief scientist, Amos Panet, and co-workers have uncovered a novel activity of recombinant reverse transcriptase. Reverse transcriptase, a key enzyme in the replication cycle of the human immunodeficiency virus (HIV-1), is a primary target for antiviral therapies. Based on Panet's finding, BTG is embarking on a drug discovery programme.

Panet's results are the outcome of a project initiated in 1988 under a research contract with the National Institutes of Health. His research with the HIV reverse transcriptase enzyme, presented at an NIH meeting in San Diego in early November 1991, suggests that it contains a third distinct enzymatic activity, ribonuclease (RNase) D, in addition to its previously known DNA polymerase and RNase H activities. RNase D cleaves HIV RNA in a complex with transfer RNA for lysine at a specific nucleotide sequence location, explains Panet.

This activity is different from RNase H, which degrades kNA in RNA/DNA complexes so that double-stranded proviral DNA can be formed and incorporated into an infected cell's genetic material. Most existing anti-HIV therapies use nucleotide analogs, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (DDC), and 2',3'-dideoxyinosine (DDI), as a means to interfere with reverse transcriptase enzymatic activity and halt viral replication. Because most cells also have such activity, an inhibitor must preferentially interact with the HIV enzyme in order not to be cytotoxic.

To be a valuable, even viable, target for drug development, this new enzymatic activity must be specific to HIV. To show this, Panet and co-workers have looked at RNase D activity in recombinant, but not virion, HIV reverse transcriptase preparations obtained from different laboratories. "A major concern at the beginning was that perhaps we were measuring a

contaminant from the [recombinant] bacteria", he says. "But we have gone through a lot of controls ... and found that the activity is inherent to the viral protein".

The enzymatic activity also must play a crucial role in the replication and life cycle of the virus. RNase D activity has been found for the murine leukaemia virus reverse transcriptase against its own specific RNA complex, explains Panet, and further studies are being conducted with avian myeloblastosis virus reverse transcriptase. (Abstracted with permission from *Chemical and Engineering News*, 16 December 1991, pp. 9 and 10. Copyright (1991) American Chemical Society)

A cervical cancer suspect

Scientists have discovered the nasty tricks used by a virus that causes genital warts. Largely because of these discoveries the virus has emerged as the primary suspect in nearly all cervical cancer.

Researchers say they have found after several years of study that the virus invades healthy cells, chemically manipulates them into dividing and then steals the substances the cells make in division to make copies of itself.

Most of the time, researchers say, the virus merely steals enough of the substances, proteins and enzymes, to make a few copies of itself. Apparently, scientists say, the immune system somehow keeps the wart virus in check.

But scientists have discovered that if this control lapses, the virus becomes more aggressive and causes the growth of warts, which in turn shed new copies of the virus. In extreme cases, the virus can invade normal cells and transform them into cancerous cells.

Based on knowledge of how the virus works, researchers said that they should be able to develop a vaccine against the virus.

Experts estimate that at least 90 per cent of all cervical tumours contain the wart virus. Although the virus does not act alone in producing cancer, Dr. Tom Broker of the University of Rochester Medical Center said it is the primary factor in the disease. In the other cases, he said, cells undergo mutations that affect the same cellular machinery co-opted by the virus. Almost every animal on earth is plagued by warts, he said, including sperm whales, beavers, cows, dogs, snakes, turtles, frogs and birds.

The viruses are highly specific. A virus that infects dogs will not infect cats, for example. Each species of animal can be infected by several strains of a virus that are defined by slight variations in the DNA building blocks.

Humans are afflicted by 70 strains of wart viruses, each of which specializes in one type of epithelium, the cells that form the skin and that line the mouth, respiratory tract, genital tract and other body surfaces.

Thus, while one will cause warts only between the fingers, another affects skin near the fingernails and another the palm, Dr. Broker said. Different strains invade the face, bottoms of the feet, or arms. An estimated 20 to 30 per cent of adults are infected with sexually transmitted wart viruses, Dr. Broker said, but most suffer no ill effects because their immune systems hold the virus in check.

Three to 4 per cent of women infected by the virus develop abnormal cervical cells, Dr. Broker said, but most of these abnormalities go away by themselves or after medical treatment. In other women, the abnormal cells are transformed into cancer cells, producing invasive tumours of the cervix.

Unlike the herpes virus, which brings tools for copying itself when it invades a cell, the wart virus does not have the basic machinery needed to replicate itself. Instead, it has to trick the cell into providing the materials for making new viral particles.

When a wart virus lands on a healthy epithelial cell, it remains harmlessly on the cell surface. But when the cell has suffered an injury, the virus can enter cells. (Source: *International Herald Tribune*, 23 January 1992)

Antibody proved to prevent HIV infection in apes

A chimpanzee has been protected from HIV infection with a specific antibody that blocks the virus. The experiment, by researchers in the United States, has focused the search for an AIDS vaccine by demonstrating for the first time exactly what part of the immune response is important.

A team led by Emilio Emini at Merck Sharp & Dohme's research laboratories in Philadelphia has developed a purified, specific antibody to part of HIV's protein coat known as the V3 loop. This loop, a string of about 30 amino acids, plays an important role in enabling the virus to enter cells. Perhaps because it is strategically so important, the loop is highly variable from one strain of HIV to another. This helps to disguise it from the immune system.

The same mechanism should apply to HIV infection in humans, though Emini will not say if any trials are yet foreseen. The drawback with their antibody Cβ1 is that it is specific to the first laboratory strain of HIV which is not typical of most strains. (Source: *New Scientist*, 22 February 1992)

Newcastle Disease virus genes

Another commercial application of genetic engineering was brought a step nearer fruition as British Technology Group plc announced that Nippon Zeon Co. Ltd. of Tokyo has signed a licence for the genes which induce protective immunity against Newcastle Disease in intensively-reared poultry. This highly infectious disease, which is also known as fowlpest, is associated with high mortality and morbidity rates - and consequently previous outbreaks have had a devastating effect on the poultry industry. The genes, which were isolated from the Newcastle Disease virus (NDV), will be inserted by Nippon Zeon into the more benign vaccine virus, known as fowlpox. The resulting recombinant vaccine will be capable of eliciting a protective immune response to infection by either NDV or the fowlpox virus. In addition, this NDV/fowlpox virus vaccine has the capacity to integrate other suitable genes at a later date, offering the prospect of a single vaccine capable of protecting poultry against a number of pathogens. In the United States, Nippon Zeon is proceeding with the development and registration of the vaccine with Syntro Corp., a US biotechnology company specializing in the development of animal vaccines and selected human health care products. Nippon Zeon is planning to establish a joint venture with Syntro to manufacture and market this innovative poultry vaccine.

Newcastle Disease occurs throughout the world and is classified as a notifiable disease. In a major UK outbreak in 1984, 800,000 birds were compulsorily slaughtered. Voluntary vaccination programmes are being used in the United States, Japan and Europe. In the United Kingdom, all chicks intended for breeding, or for egg production for human consumption, are routinely vaccinated.

Current vaccines are produced from inactivated or live strains of NDV. There are drawbacks, however, with both these forms of vaccination. Inactivated vaccines are expensive to produce. Live vaccines vary in their effectiveness and give rise to adverse effects, e.g. reduced egg production and mortality in susceptible chickens. In contrast, live strains of fowlpox virus (the "vector" virus in Nippon Zeon's novel vaccine system) do not normally result in any adverse effects.

The NDV genes are complementary DNA clones of two glycoproteins associated with the viral envelope. Antibodies raised against the glycoproteins prevent: (1) the attachment of NDV to host cell receptors; (2) fusion of the virus with the cell membrane; and (3) penetration by the virus into the host cell. The genes for these immunity-stimulating glycoproteins were isolated in 1985 by Professor Peter Emmerson at the University of Newcastle upon Tyne.

Studies at the Houghton Laboratory of the FARC's Institute for Animal Health have already shown that birds vaccinated with a fowlpox vector vaccine containing just one of BTG's NDV genes are resistant to a virulent disease-causing strain of NDV. Details from: British Technology Group plc, 101 Newington Causeway, London SE1 6BU, or on 071-403 6666. Fax: 071-403 7586. (Source: *Biotechnology Bulletin*, March 1992)

Universal Virus Data bank (UVDB)

The International Committee on Taxonomy of Viruses (ICTV) agreed last year on the definition of a viral species, and is now catalysing and promoting an ambitious global project for a data bank spanning the whole field of viruses - animal and human, plant, insect, bacterial or other (including viroids). The project builds on 60 man-years of Australian Centre for International Agricultural Research; but is now incorporated in the UVDB concept, broader both scientifically and geographically. ICTV is run by the Virology Section of IUMS, the International Union of Microbiological Societies of ICSU, the International Council of Scientific Unions.

On 24 February 1992 key members of ICTV were in Brussels to inform Commission staff about the project; for further details, contact either CUBE or the following participants:

Prof. Adrian Gibbs, Australian National University, PO Box 475, Research School of Biological Sciences, Canberra, ACT 2601, Australia. Tel.: (61) 62-49-4211; Fax: (61) 62-49-4437;

Dr. Claude Fauquet, the Scripps Research Institute, Division of Plant Biology - MRC7 10666, North Torrey Pines Road, La Jolla, CA 92037, USA. Tel.: (1) 6195542906; Fax: (1) 6195546330;

Dr. Gunter Adams, c/o Biologische Bundesanstalt, Messeweg 11/12, D-3300 Braunschweig, Germany. Tel.: (49) 531399645; Fax: (49) 531399239;

Dr. A. Brunt, Horticulture Research International, Worthing Road, Littlehampton, West Sussex, BN17 6LP, UK. Tel.: (44) 903716123; Fax: (44) 903726780.

(Source: *EBIS*, Vol. 2, No. 1, 1992)

Research instrumentation

Antigenic peptides analysed, sequenced by mass spectrometry

An analytical technique has been developed that makes it possible to characterize peptides bound to the surfaces of antigen-presenting cells. The technique was developed and implemented by Donald F. Hunt and

Jeffrey Shabanowitz of the University of Virginia chemistry department, Robert A. Henderson of the University of Virginia School of Medicine, Kazuyasu Sakaguchi of the National Cancer Institute, and co-workers. In the immune system, antigens are recognized by cytotoxic T-lymphocytes only after they have been processed by other cells and displayed as short peptides on the cell surface. Previously, HPLC was used with Edman degradation to characterize and sequence these peptides. However, this method yielded questionable data, in part because HPLC could not fully resolve the complex mixtures of peptides that can consist of as many as 1,000 components. Now, using microcapillary HPLC and electrospray ionization tandem MS, the researchers have solved this problem by adding a second separation step. The peptides are first fractionated by HPLC into simpler mixtures. The first of two mass spectrometers then selects one of the many peptides eluted from the microcapillary column and transfers it to the second mass spectrometer, where it is fragmented and sequenced. The technique should make it possible to characterize antigens associated with viral infection, autoimmunity, and other diseases, the researchers say. (Reprinted with permission from *Chemical and Engineering News*, 16 March 1992, p. 26. Copyright (1992) American Chemical Society)

Group develops new protein sequencer

A research group from the Science University of Tokyo's Research Institute for Biosciences has developed a new method of sequencing proteins, starting from the C terminal, the opposite direction used by existing methods. The new method, which reportedly enables 10-20 amino acids to be sequenced in one hour, can reportedly be applied to proteins impossible to sequence by conventional techniques which start from the N terminal. The new technology uses pentafluoropropionic anhydride, which removes amino acids, one by one, from the C terminal of a polypeptide. Specifically, a sample of protein is placed in a small test tube and dried, and then exposed to the vapour of the above compound at -20° C for 10-60 minutes.

This removes amino acids one by one from the C terminal. The product of this reaction is a mixture of proteins with 1-20 times fewer amino acids than the original protein. The mixture is then subjected to mass spectrometry to determine the masses of the individual substances, from which the amino acid sequence can be determined.

A method of sequencing proteins from the N terminal, which was developed 30 years ago, has been automated to the point where 20 amino acids can be sequenced at a pace of two amino acids per hour. However, a functional group at the N terminal may sometimes disturb decoding, and about 50 per cent of proteins have been impossible to sequence. The new technique overcomes these difficulties and is therefore expected to have a major impact on the biotechnology industry. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

SPOTs - a novel system to map antibody epitopes

Cambridge Research Biochemicals have launched SPOTs - a novel, low-cost method for the detection of antibody epitopes. SPOTs uses a novel cellulose membrane upon which peptides may be synthesized, each one permitting 96 peptides to be made in an 8 x 12 format. To epitope scan an antibody, a complete set of overlapping peptides are generated from the antigen using the IBM software provided. Synthesis is rapid and simple, taking only 90 minutes per amino acid. SPOTs is low cost, does not require special equipment, and has a very low solvent usage. Once synthesized the SPOTs membranes are incubated with antibody just like a dot-blot. The SPOTs, which have bound antibody, turn blue after incubation with substrate, thus indicating the antibody's epitope. The SPOTs membranes may then be regenerated and probed again with antibody.

Further details from Cambridge Research Biochemicals Ltd., Gadbrook Park, Northwich, Cheshire, CW9 7RA, UK. (Source: *Press Release*, 2 April 1992)

Screening kit and media for hydrophobic interaction chromatography

Affinity Chromatography Ltd. (Isle of Man, UK) has launched a comprehensive range of 10 adsorbents for hydrophobic interaction chromatography (HIC), comprising five ligands of increasing hydrophobicity - butyl, hexyl, octyl, decyl and phenyl groups - immobilized on 4 per cent and 6 per cent cross-linked agarose. Choosing the best adsorbent for a given separation is made easy with the PIKSI-H screening kit, which comprises integral columns and reservoirs for all 10 media. This approach to HIC complements ACL's currently available adsorbents for protein purification. The robust adsorbents all withstand regeneration with molar sodium hydroxide and are available in larger individual packs for laboratory-scale separations and in bulk for process applications. (Source: *News Release*, February 1992)

General

New LBL gene map technique

Scientists at the Lawrence Berkeley Laboratory (LBL) in Berkeley, California have developed a new method for locating the positions of genes on chromosomes.

The researchers call the novel technique "random-breakage mapping", and say it is a quick and relatively easy way of mapping internal DNA sequences with respect to the ends of the chromosome.

Random-breakage mapping is best understood by considering a chromosome that is broken only once. For a once-broken chromosome, the shortest fragment that can contain the DNA sequence of a specific gene must be a piece with the gene on the end.

Restriction fragments

Dr. J. Game and his team developed the random-breakage mapping technique for their research with yeast DNA. The method cannot be used on whole human chromosomes at this time because even a once-broken human chromosome is still too large to be resolved in today's electrophoresis gels. Even if a gel could resolve such large molecules, the probe signal might be too weak.

However, once a human gene known to be on a particular chromosome has been cloned, random-breakage mapping could be used to pinpoint its location. Restriction fragments would first be used to selectively fragment the chromosome. The restriction fragments, whose position along the chromosome is known, could then be handled in the same manner as yeast chromosomes in order to map the gene. Dr. Game notes that the technique should also be well-suited to the analysis of mammalian DNA that has been introduced into yeast chromosomes for replication purposes, and to studies of DNA breakage and repair.

Dr. Game has carried out his work with LBL scientists Maren Bell and Robert Mortimer, and with Jeff King at the University of California in Berkeley. (Source: *Genetic Engineering News*, April 1992)

New type of antibody can bind two antigen molecules

Dimeric antibody fragments that are bivalent (able to bind two antigen molecules) and capable of being produced in *Escherichia coli* have been developed by Peter Pack and Andreas Plückthun of the University of Munich, Martinsried, Germany. The researchers call them "miniantibodies" because of their small size relative to native antibodies. Recombinant Fv (variable) fragments and larger Fab (antigen-binding) fragments have been produced previously in *E. coli* and shown to bind antigen effectively, but all such fragments have been monovalent, able to bind one antigen molecule only. Construction of a miniantibody starts with a single-chain Fv antibody fragment - one light- and one heavy-chain variable domain linked together covalently. To the Fv fragment is appended a "hinge" sequence, which adds conformational flexibility; an amphiphilic helix, which induces dimerization; and an optional hydrophilic tail containing cysteine, which forms a disulphide bond upon dimerization. The fragments are produced by recombinant techniques in *E. coli*. Bivalent binding by miniantibodies results in substantially stronger complexes than binding monovalent fragments. Miniantibodies presumably could also be designed to bind two different antigens at the same time. Such heterobifunctional antibodies could be useful in cancer therapy - for example, by combining affinity for a tumour market with the ability to bind an immune-system cell likely to attack the tumour. (Reprinted with permission from *Chemical and Engineering News*, 2 March 1992, p. 22. Copyright (1992) American Chemical Society)

Microbes in space

Bacteria proliferating in the microgravity environment of space grow at the same rate, and with the same energy efficiency, as those on earth, according to experiments designed by Philippe Bouloc and Richard d'Ari of the Jacques Monod Institute at the University of Paris in France. Their observation on cultures of *Escherichia coli* carried on the Soviet satellite Bicosmos 2044, orbiting the earth, repudiates earlier suggestions that earth-bound micro-organisms have to expend significant amounts of energy in fighting against the effects of gravity and can therefore grow more efficiently or rapidly in space.

Though disappointing for exobiotechnologists who hoped to achieve increased bioefficiency in orbiting bioreactors, the finding has a more welcome implication. If it is applicable to pathogenic bacteria too, the discovery militates against anxieties that astronauts face increased dangers of infection. Furthermore, Bouloc and d'Ari report that the cosmic radiation which penetrated into the satellite did not cause significant levels of damage to the *E. coli* DNA. Radiation hazards for both bacterial and human inhabitants of space may be less than was thought. (Extracted from *Bio / Technology*, Vol. 10, February 1992)

Genetic code gains a new letter

The genetic "alphabet" has been expanded artificially, improving the prospects of making new proteins. Researchers in Switzerland and California have synthesized a new genetic "instruction", or codon, of genetic material. They have inserted the codon into RNA, the molecule into which DNA is copied during protein synthesis, and persuaded it to translate into a novel amino acid that can slot into a protein chain.

The team says this is the first of three breakthroughs that will be needed if synthetic amino acids are to be incorporated in living cells. The achievement will excite biotechnologists and the pharmaceuticals industry because it raises the possibility of "designer" proteins in future.

Steven Benner at the Swiss Federal Institute of Technology in Zurich, and his colleagues at the University of California, have been working on this problem for some time. Two years ago, they showed it was possible to make an artificial base pair in DNA and

RNA, so proving the genetic alphabet could be extended in principle.

The team says there are two further hurdles to be cleared before biotechnologists will be able to control the making of new proteins inside the body. First, stretches of DNA with altered base pairs must be copied and transcribed faithfully in an organism. Secondly, it will be necessary to engineer special forms of the enzyme RNA synthetase, to couple novel amino acids to novel transfer RNA. This will be more difficult. If these difficulties can be overcome, the technique could be applied to many processes in biotechnology. For example, Benner has suggested that RNA molecules could be made more powerful to catalyse reactions, such as their own replication. (Source: *New Scientist*, 11 April 1992)

How "mutant" molecules fight for survival

Chemists in the United States believe they have discovered the chemical equivalent of biological selection. The team, led by Julius Rebek of the Massachusetts Institute of Technology, have carried out experiments with two self-replicating molecules, and discovered that they can cooperate, catalysing each other's formation. Furthermore, when one of the molecules is exposed to ultraviolet light and is "mutated", it becomes "aggressive" and takes over the system. According to Rebek and his colleagues, this is evidence that evolution can be modelled at the molecular level. (Extracted from *New Scientist*, 29 February 1992)

Porphyrins provide the key to "designer enzymes"

Chemists in the United Kingdom have designed and built "supermolecules" from compounds common in nature. They hope these will enable them to produce efficient catalysts, capable of mimicking enzymes, the biological catalysts.

Like enzymes, the supermolecules have an "active site" - a cavity or cleft - in which they can trap a "substrate" molecule, speeding up any chemical reactions in which it is involved. However, enzymes have "wasteful" molecular scaffolding surrounding the active site, and a lot of this can be dispensed with in the designed molecules.

Jeremy Sanders and his colleagues at the University of Cambridge have been using derivatives of porphyrins, compounds common in nature, to make their supermolecules. They use the technique of "template synthesis", which exploits the ability of some molecules to "self-assemble", or join together spontaneously when guided by a template molecule.

A good example of the template effect is the formation of crown ethers. When the components of a cyclic ether are mixed with an appropriate alkali metal ion (for example, lithium), they wrap themselves spontaneously around the template ion to form a ring of a specific size. The size depends on the ion. Lithium provides a template for the formation of 12-crown-4 ($C_8H_{16}O_4$), for example.

Sanders and his colleagues made their supermolecules by incorporating zinc atoms into the centres of porphyrin molecules, then joining pairs of these to form "dimers". They then used the template effect to carry out a further controlled coupling, this time using tris (pyridinylacetoacetate) aluminium as the template.

The researchers believe that during this step the porphyrin dimer wraps itself around the triangular aluminium-containing template, bringing the reactive ends close so that a third porphyrin can couple to complete the ring.

The resulting supermolecule - a cyclic trimer with an aluminium template held in the centre - has the amazing formula $C_{210}H_{246}P_6O_6Pt_3Zn_3Al$. According to Sanders, very few molecules have quite as many different elements.

The chemists released the trimer from the template molecule by removing the zinc atoms from the centre of each porphyrin ring. The resulting ring has a cavity about 0.18 nanometres in diameter.

The size of the cavity determines which substrate molecules will fit inside the ring, and so is important in finding catalytic applications. Sanders' group is investigating the potential of these molecules.

Sanders and his colleagues are also building supermolecules using the steroid cholic acid. They have made cyclic polymers "cyclocholates" with large water-attracting, or hydrophilic, cavities. The outside of the molecule is hydrophobic or oily. Such molecules could be used to transport hydrophilic drugs across cell walls, acting as selective agents to deliver drugs.

By combining porphyrins with the cyclocholates, the researchers have made a steroid-capped porphyrin which they believe also has promise as a catalyst. (Source: *New Scientist*, 1 February 1992)

Cloned haemoglobin genes enhance cell growth

Experiments have shown that portable deoxyribonucleic acid (DNA) sequences can be incorporated into host cells to make them produce haemoglobins - oxygen-binding proteins essential to the function of red blood cells. This biotechnological feat is accomplished by a recombinant-DNA method that includes the following essential steps:

- Preparation of part of a gene or other natural or artificial DNA sequence capable of directing the host cell to produce haemoglobin;

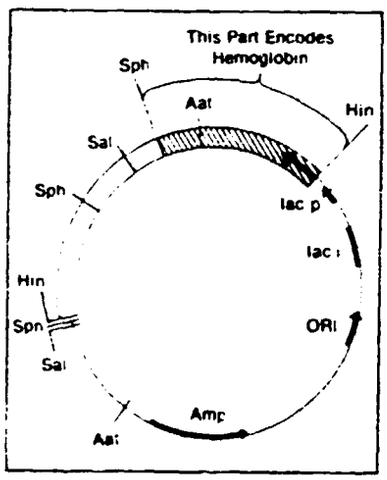
- Cloning the portable DNA sequence directly into the host cell or into a vector (e.g., a plasmid or virus) that can be transferred into, and be replicated in, the host cell;

- Transferring the vector (if used) into the host cell;

- Culturing the host cell under conditions that favour the replication and propagation of the vector and the production of the haemoglobin; and

- Harvesting the protein and activating it to bind oxygen.

The host cells used in the experiments were *Escherichia coli* bacteria. The portable DNA sequence chosen for the experiments was part of the genetic material of the bacterium Vitreoscilla, which produces a type of haemoglobin. The vectors were pUC19/pRED2 and other plasmids of the *E. coli* cells (see figure).



This **Nucleotide Sequence** of the plasmid pUC19/pRED2 of *E. coli* includes a part from Vitreoscilla that encodes the production of haemoglobin.

The vectors were chosen on the basis of their ability to cause the host cells to express the portable DNA sequence. In particular, a vector should contain "operational elements" - DNA sequences that express promotion, regulation and termination of the production of haemoglobin. Finally, DNA sequences for transcription and translation should be present.

One set of experiments yielded recombinant *E. coli* cells that included significant amounts of haemoglobin, indicated by a reddish tint. In another set of experiments, the recombinant *E. coli* cells were found to grow faster and to a greater density than did unaltered cells of the same strain grown under identical conditions. In a third set of experiments, it was observed that the production of haemoglobin the recombinant cells decreased with an increase in the oxygen content of the culture medium.

The method should be useful in several biotechnological applications. One is the enhancement of growth of cells at higher densities. Another is the production of haemoglobin to enhance supplies of oxygen in cells, for use in chemical reactions that require oxygen, as an additive to serum to increase the transport of oxygen, and for binding and separating oxygen from mixtures of gases.

This work was done by Chaitan Khosla and James E. Bailey of Caltech for NASA's Jet Propulsion Laboratory. Further information may be obtained from Edward Ansell, Director of Patents and Licensing, Mail Stop 305-6, California Institute of Technology, 1201 East California Boulevard, Pasadena, CA 91125. (Source: *NASA Tech Briefs*, January 1992)

Environmental control of a genetic process

Experiments have shown that the environment can be manipulated to control such genetic processes as the transcription and translation of DNA sequences. In particular, *E. coli* bacteria altered to contain a DNA sequence that encodes the production of haemoglobin were made to produce haemoglobin at rates that decreased with increases in the concentration of oxygen in the culture media.

Here, the emphasis is on some of the DNA sequences that usually precede the gene of interest in a DNA polymer. Among these are the "promoter" sequences, which provide sites for the initiation of transcription of the gene into messenger ribonucleic acid. Others, called "regulator" sequences, include attenuators, regulators, and enhancers, which determine the frequency or rate of initiation of transcription and/or translation. The combined effect of all of these, known collectively as "promoter/regulator" DNA sequences, is to determine the extent of eventual expression of the gene.

There is enormous variety in the structures and functions of promoter/regulator sequences. Functions can be activated or deactivated by various environmental factors, including changes in temperature and the presence or absence of various substances. In this case, the experimenters sought a promoter/regulator sequence that would switch from low to high expressive activity at constant temperature upon the reduction of the concentration of oxygen dissolved in the culture

medium. Such a promoter/regulator sequence would be advantageous in a laboratory setting because it is easy to reduce the level of dissolved oxygen at high cell densities, without having to add any chemicals to the growth medium to induce the expression of genes.

The recombinant-DNA concepts and techniques used to demonstrate the manipulation of promoter/regulator DNA sequences were similar to those described in the preceding article. As before, DNA sequences from *Vitreoscilla* (strictly aerobic bacteria that live in oxygen-poor environments) were implanted in the *E. coli* bacteria via plasmid vectors. The "upstream" (in a genetic sense) promoter/regulator sequence was fused with a "downstream" sequence that encodes haemoglobin.

The manipulation of promoter/regulator DNA sequences opens a promising new subfield of recombinant-DNA technology for the environmental control of the expression of selected DNA sequences. New recombinant-DNA fusion gene products, expression vectors, and nucleotide-base sequences will emerge. Likely applications for this new technology include such aerobic processes as the manufacture of cloned proteins and synthesis of metabolites, the production of chemicals by fermentation, enzymatic degradation, the treatment of wastes, brewing, and a variety of oxidative chemical reactions.

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Glycobiology tackles old problems in new ways

Glycobiology - the study of the role of sugars in biological processes - is a newly-emerging area of science just beginning to attract the interest of the pharmaceuticals industry. Yet it is already showing new approaches to the treatment of some of mankind's most stubborn diseases, and could rival some of the better known areas of biology in the quest for new drugs.

The term was coined five years ago by Professor Raymond Dwek, head of Oxford University's Institute of Glycobiology, a collaborative research centre with the US pharmaceuticals group Searle. In fact Searle, and its parent company Monsanto, are amongst the first to recognize potential for drug discovery via glycobiology.

The Institute of Glycobiology, whose new laboratories were opened recently, is the largest of only a handful of scientific groups working in this area in the world. It houses some 60 researchers, including scientists from Searle's US laboratories, who concentrate on applying research findings to the development of novel therapeutic agents.

The Institute's work centres on the many proteins involved, in particular, with immunological diseases, which are glycosylated, that is coated in oligosaccharides or sugar molecules. Generally, for the same glycoprotein, a number of different glycoforms exist, as the number and position of the individual oligosaccharides can vary. The Institute's research is based on the observation that many protein glycosylations have a fundamental role in many cell biochemical processes. Glycosylation seems to extend the range of properties of the protein, say for example the immune system messengers or cytokines, and can within a given environment determine or control its function. Furthermore, a change in the relative population of the different glycoforms appears to be associated with some disease types, either directly as a result of the pathogenic process or in response to it.

The crux of the work is that modification of the glycoform population offers a potential pharmacological point of intervention. Because of the typical involvement of highly glycosylated proteins, glycobiology offers the most potential in the area of immunology.

Oligosaccharides attached to proteins and those exposed to the surface of cells can modify the immune system response in a number of ways. These include the blood group sugars (ABO and rhesus), which can cause fatal transfusion reaction if blood is injected into incompatible donors, while oligosaccharides coating the surface of tumour cells help to protect these cells from detection and destruction by the host's immune system.

Much of the research is at an early stage, and the elucidation of the case-effect relationship of disease with different glycoform populations will provide insights into how such diseases could be treated. None the less, this "knowledge gap" has not prevented the discovery of a novel drug currently in trials as a treatment for AIDS.

The drug, *Oxaid* (butyl-DNJ), is in development by Searle, as an adjunct treatment to zidovudine in HIV infection.

The three other projects at the Institute are less advanced, and aimed at elucidating the function of glycoforms in asthma, rheumatoid arthritis and pre-eclampsia (toxaemia in pregnancy).

Longer term, it is hoped that the application of structural and functional information gained from investigation into the role of cell surface sugars, can be used in the development of sugar mimics which may modify the immune response in other situations. These could include immunosuppressive agents, to prevent organ transplant rejection, and immunomodulators, compounds that can augment the body's immunological defence mechanism for destroying cancer cells. (Extracted from *European Chemical News*, 23/30 December 1991)

D. APPLICATIONS

Pharmaceutical and medical applications

Two gene-therapy trials

Genetic Therapy Inc. said the United States' National Institutes of Health approved two gene-therapy trials and two gene-transfer trials for treatment of cancer. One trial, for the treatment of primary brain tumours, is designed to induce cancerous cells to produce an enzyme that makes tumours susceptible to the anti-viral drug *Ganciclovir* the US biotechnology concern said. The NIH also approved a gene-therapy trial involving the use of Interleukin-2, which induces immune-cell growth in the treatment of neuroblastoma, the most common form of solid tumours in children. The trials will begin after approval by the US Food and Drug Administration. (Source: *Wall Street Journal*, 5-6 June 1992)

Transplant rejection suppressed

Research by Dr. Mitsuaki Isobe of the Faculty of Medicine at the University of Tokyo (Japan) has yielded a novel approach to suppressing the rejection of organ transplants. Isobe's method entails the injection of two monoclonal antibodies, anti-ICAM1 and anti-LFA1, immediately after the transplant operation to suppress the action of the recipient's T-cells. The antibodies prevent binding between surface molecules on the recipient's T-cells and the surface of the organ's cells. In experiments, Isobe injected the monoclonal antibodies into mice bearing a mouse heart transplant in the abdominal region.

According to the results, the heart was not rejected in any of the animals. Isobe says that the potent efficacy of his dual antibody administration suggests that intraspecies transplantation may be possible if his new method of immunosuppression is used. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Breast cancer drug goes on trial in US

Healthy women in the US and Canada are soon to begin a trial of the drug *Tamoxifen* to see whether it prevents breast cancer. The study, which will involve 16,000 women, could be under way before a similar trial in the UK, which has become entangled in a controversy over the drug's safety.

Samuel Broder, director of the National Cancer Institute in the US, said the trial was important because it might demonstrate a "practical method" of preventing breast cancer in women at high risk. Half the women in the trial will take *Tamoxifen* daily for five years; the other half will take a placebo.

Tamoxifen is effective in treating breast cancer and in reducing the risk of a woman developing a second tumour in her opposite breast. However, it also increases the risk of cancer of the lining of the womb and may slightly increase the risk of blood clots.

Researchers at the NCI expect the incidence of breast cancer will be reduced by about one third in the 8,000 women receiving the drug. This amounts to 62 cases of cancer prevented. At the same time, the drug is expected to cause 38 cases of cancer of the lining of the womb, none of which should prove fatal, and 3 deaths from thrombosis. (Extracted from *New Scientist*, 9 May 1992)

Malaria vaccine trials

Spain has approved an experimental malaria vaccine for trials in people, just months after Britain's Medical Research Council rejected it. The move renews controversy over the vaccine and increases the pressure on the MRC to explain its decision.

At present, no widely approved vaccine exists for malaria, which causes disease in about 130 million people and kills 2 million a year. The experimental vaccine was developed by a Colombian scientist, Manuel Patarroyo, and has been tested in 20,000 people in Latin America. Patarroyo claims it works, but other scientists and the WHO say that independent trials of the vaccine are urgently needed.

Last December, the MRC decided for the second year running not to authorize a trial of the vaccine by its own researchers in The Gambia, West Africa, saying that it needed more information about the composition of the vaccine and its variability between batches.

The Spanish Ministry of Health, whose approval procedures differ from Britain's, cleared the vaccine. A trial is now planned in Tanzania with the cooperation of the Tanzanian Government and the Swiss Tropical Research Institute.

Patarroyo's vaccine is based on a string of synthetic peptides that mimics the parasite's surface proteins. When these peptides are assembled into a polymer they may vary from batch to batch and there is a risk of minor changes in the structure. John Horton, head of therapeutics in tropical medicine for SmithKline Beecham, says he doubts that enough data is yet available on the vaccine to allow it to be registered, but he adds that trials of vaccines are often begun before all data are available. (Extracted from *New Scientist*, 22 February 1992)

Artificial blood

A British laboratory has developed an artificial form of haemoglobin, the red protein in blood that carries oxygen. Trials of the protein, which should work

as a safe substitute for blood, will begin shortly in the US.

Kiyoshi Nagai and his colleagues at the Medical Research Council's Laboratory of Molecular Biology in Cambridge are working with the American company Somatogen because no British company was interested.

The team's haemoglobin is made by inserting the genes for the protein into the bacterium *Escherichia coli*. Previous attempts to make artificial haemoglobin have failed because the protein breaks down rapidly and its breakdown products may damage the kidney. Also, the artificial protein clings onto its oxygen rather than allowing the oxygen to move into tissues.

Nagai's team has linked the chains of the protein together so that it does not break down. The team has also introduced a mutation into one of the genes so that the artificial protein gives up its oxygen more easily. (Source: *New Scientist*, 21 March 1992)

Big step towards cystic fibrosis gene therapy

Scientists at the National Institutes of Health have developed a potential cure for cystic fibrosis, although its safety and effectiveness in humans have yet to be tested.

The researchers at the National Heart, Lung & Blood Institute (NHLBI) used a genetically altered cold virus as a carrier to insert a normal copy of the human cystic fibrosis gene into cells lining the lungs of laboratory rats. Once implanted there, the gene instructed the lung cells to manufacture the protein that, when defective in humans, causes the fatal disorder. The group describes its findings in the 10 January issue of *Cell*.

Since an animal model for cystic fibrosis is not known, the efficacy of this approach cannot be tested in animals, but earlier test-tube experiments showed that inserting normal copies of the cystic fibrosis gene into lung cells obtained from patients with the disease corrects the abnormality. So this approach should work in living patients.

Some concerns remain, however. For instance, what if the weakened adeno-virus becomes virulent again? What if it invades tissues other than the lungs and produces the CFTR protein there? Could the human immune system attack the engineered viruses, rendering them useless for gene therapy? (Abstracted with permission from *Chemical and Engineering News*, 20 January 1992, p. 6, article written by R. Dagani. Copyright (1992) American Chemical Society)

Mab against rheumatoid arthritis

Large numbers of people suffer from rheumatoid arthritis all their lives. When the illness develops, what are known as T-lymphocytes, the otherwise reliable scavenger cells that help combat infections, attack certain tissue structures inside the body instead of invaders from outside. They do this for no apparent reason, but it is known that the phenomenon is caused as a result of stimulation by the CD 4 molecule. Scientists at the University of Erlangen-Nüremberg have now developed an "antidote": a monoclonal antibody with the name Max 16H5. It blocks CD 4 and thus stops the inflammation. Two-year clinical tests were favourable. The complaint disappeared in three quarters of the patients. The side-effects were tolerable; fever and sickness only occurred in the first few days. (Source: *Scala*, February 1992)

GLUT-2 gene opens road to artificial pancreas

Researchers at the University of Texas Southwestern Medical Center say they have developed a way of making genetically engineered cells secrete insulin only when the body of a diabetic patient needs it. The breakthrough promises to overcome a major stumbling block to the development of an artificial pancreas.

The artificial pancreas consists of insulin-producing beta cells, extracted from an animal pancreas gland, encapsulated in a 2.5 cm-long, wire-thin plastic cylinder. Successful animal tests of such systems were reported in December 1992 by two different research teams, one at Washington University at St. Louis, working with CytoTherapeutics Inc. of Providence, Rhode Island, and the other at BioHybrid Technologies Inc. of Shrewsbury, Massachusetts.

The Texas work involved taking mouse pituitary cells, genetically engineered in 1983 to make human insulin, and inserting the GLUT-2 gene - which lets glucose seep into the cell and trigger the secretion of insulin. The mouse cells thus mimic the beta cells of the pancreas in regulating insulin output in response to the level of glucose in the blood.

The cells still need further work: they begin to secrete insulin too early, for example, and they also secrete a mouse version of a pituitary hormone, ACTH, which may - or may not - affect humans. But the team leader, Christopher Newgard, assistant professor of biochemistry at the university medical centre, believes these roadblocks can be readily overcome. (Source: *Biotechnology Bulletin*, February 1992)

US poised to approve Interleukin-2

An advisory committee has recommended that the US Food and Drug Administration (FDA) approve Interleukin-2 (IL-2) for treating a lethal form of kidney cancer.

Even though this potent protein molecule does not "meet the conventional definition of safe and effective", committee members concluded, "a small group of patients stands to gain significantly". The committee also said that if the agency approves the product, it should be accompanied with information describing toxic effects and current limits to effectiveness.

This is the second time the committee has reviewed IL-2, which is known commercially as *Proleukin*. The product is now sponsored by California-based Chiron, which recently merged with the product's original corporate sponsor, Cetus.

IL-2 still appears only marginally effective against kidney cancer. However, no other conventional treatment is considered effective against this rare but deadly disease which kills, on average, within six months to a year.

Overall, 15 per cent of patients treated with IL-2 showed a positive response, with 4 per cent showing what was deemed a "complete response".

Treatment with IL-2 seems to shrink malignancies in some patients. However, the treatment is associated with "severe toxicities", including an "on-study death rate of 4 per cent". Side-effects include nausea, hypotension, renal toxicities, neurological effects and sepsis. But in most cases, with proper and close patient management, those adverse effects are reversible, says Chiron. (Source: *Chemistry & Industry*, 3 February 1992)

Broccoli may protect against cancer

Researchers have isolated and identified a key chemical in broccoli (*Brassica oleracea*) that strongly induces enzymes that protect against cancer. People who consume broccoli and other cruciferous vegetables, such as cauliflower, mustard, and brussels sprouts, are known to have a lower risk of developing cancer. The protective effect is thought to be due, at least in part, to compounds in these vegetables that induce so-called phase II detoxication enzymes, which are involved in the metabolism of carcinogens. Researchers have found that most of broccoli's enzyme-inducer activity resides in a single chemical known as *sulphoraphane*. This compound's presence in plants has been known for over three decades, and related isothiocyanates are known to have anticarcinogenic properties. But of all of the selective phase II enzyme inducers found in vegetables, *sulphoraphane* is so far the most potent. Scientists are now eager to test the compound to see if it will block tumour formation in animals. (Abstracted with permission from *Chemical and Engineering News*, 30 March 1992. Copyright (1992) American Chemical Society)

Major tumour response observed with BC-IL cancer drug

Ongoing phase II clinical trials of the immune boosting drug BC-IL for terminal head and neck cancer patients have shown significant reduction of tumour volume estimated to be considerably greater than 50 per cent in two patients treated. The world-wide rights to BC-IL, a natural lymphokine "cocktail", are owned by Cel-Sci Corp. (Alexandria, Virginia, USA).

The first two patients had previously received radical surgery followed by radiation therapy. However their health continued to deteriorate. Both patients had low levels of T-cells and evidence of immune deficiency, generally a characteristic of head and neck cancer.

The BC-IL treatment was administered on an out-patient basis with negligible side-effects. Both patients showed an initial doubling of blood T-cell counts reflecting a potent stimulation of their immune systems. They also showed lymphocyte infiltration into the tumour sites indicating an intense immune reaction to the tumour. The first patient has maintained tumour reduction over three months. The second patient has just completed his first month. (Source: *BFE*, Vol. 9, No. 1-2, January/February 1992)

Possible new treatment for skin cancer

A possible new treatment for skin cancer using a combination of ultraviolet light and titanium oxide has been developed by Akira Fujishima and Kazuhito Hashimoto of Tokyo University and Yoshinobu Kobuta of Yokahama University. The researchers transplanted cancer cells from a human uterus in mice. Titanium oxide was injected into the tumours of half the mice when the tumours grew to some 8 mm. Subsequent application of ultraviolet light to the skin tumours virtually eradicated the cancer cells after one month. The researchers say their treatment has none of the side-effects associated with traditional cancer treatments. (Extracted from *Asian Wall Street Journal*, 18 May 1992)

AIDS/AZT early treatment

A new clinical study shows that early treatment with Wellcome's AIDS drug, AZT, can slow the progress of the disease, but does not affect survival rates. Early treatment with AZT was also associated with more side-effects.

The new information is likely to rekindle an ongoing debate: at what stage in the disease should AZT treatment begin? Another recent study showed that AZT treatment could slow progression of the disease in otherwise healthy patients who carry the HIV virus.

The latest report, from a group headed by John Hamilton at Duke University in North Carolina, focused on HIV-positive patients who already exhibited some symptoms of AIDS. Of the 338 patients in the study, half received AZT from the outset. The others began the treatment when the number of CD4 immune cells fell below the level of $200/mm^3$, or when full-blown AIDS developed.

"Early zidovudine (AZT) therapy slowed the progression to AIDS but did not improve overall survival in our trial", the researchers say. (Source: *Chemistry & Industry*, 2 March 1992)

EscaGenetics offers taxol

EscaGenetics Corporation, San Carlos, California, is supplying taxol produced by its proprietary cell tissue culture technology to the National Cancer Institute for its research in curing ovarian cancer. Using fermentation technology, EscaGenetics produces taxol from cell cultures consisting of root, needle and stem cells of Pacific yew trees.

This technology represents a breakthrough in taxol production because traditional methods require manufacturers to strip the bark of four to six mature yew trees in order to extract one to two grams of taxol, the amount required to treat one patient.

In addition, by using its "PhytoProduction" cell tissue culture technology, EscaGenetics is producing higher concentrations of taxol than yew bark, a capability that may eliminate the need to sacrifice entire tree stands. (Source: *Chemical Marketing Reporter*, 30 March 1992).

Pilot trial for blood clot detector

Bio-Technology General has started pilot clinical trials on a radio-imaging agent capable of detecting blood clots. The company believes its techniques will provide a precise and reproducible non-invasive detection of blood clots.

BTG has developed a genetically modified fibronectin fragment which specifically binds to fibrin, a protein found only in blood clots. The convenience and rapidity of clot detection by this agent could permit early medical intervention in deep vein thrombosis (DVT) and pulmonary emboli patients and reduce both mortality and morbidity, the company claims.

Conventional DVT diagnosis relies on invasive procedures which can lead to side-effects or non-invasive methods that detect thrombi indirectly but are not too sensitive in DVT detection and may give false negative diagnoses. (Source: *European Chemical News*, 16 December 1991)

Immunotherapy

In the struggle against cancer, AIDS, rheumatism, multiple sclerosis and allergies, doctors all over the world are increasingly placing their hopes in a new approach: immunotherapy, treatment aimed at strengthening the body's own defences and reducing physical malfunctions. "A real gold-rush atmosphere" now prevails among immunologists and genetic engineers. However, as yet none of the researchers is prepared to speak of a great breakthrough - there are still only promising beginnings, for example, at the teaching hospital in Erlangen where intensive work has been carried out on T-cells. Normally these cells represent the immune system's active defences. They investigate suspicious objects inside the body for "immunological tolerance" and mobilize scavenger cells (macrophages) to attack viruses and bacteria. Often, however, they overdo things and give the order to assault when there are no intruders in sight. After being activated in this way, the macrophages will then simply attack nerves and joints due to a lack of real enemies. This results in multiple sclerosis or rheumatism. As yet no one knows why the T-cells suddenly get out of control. Nevertheless, the researchers in Erlangen succeeded in immobilizing wild T-cells with antibodies. The new therapy is already being given to patients suffering from rheumatism. (Source: *Scala*, January-February 1992)

Mouse cells engineer hope for diabetes treatment

A tiny plastic tube filled with cells genetically modified to produce insulin could one day form the basis of an artificial pancreas to treat diabetes. American biologists have claimed a breakthrough in developing such a device.

Christopher Newgard and his colleagues from the University of Texas at Dallas say they have modified a cell from the pituitary gland of a mouse to act like a pancreas cell, secreting insulin when stimulated by glucose. According to Roger Unger, director of the diabetes institute at Dallas, this marks "the first step in designing a cell that meets the needs of a diabetic". One of the main aims of diabetes research has been the quest for an implant containing cells to produce insulin in the body.

The advance, reported in the *Proceedings of the National Academy of Sciences*, follows a report of a trial in mice of a small hollow fibre designed to hold insulin-releasing cells and protect them from attack from the recipient's immune system. The researchers have patented their modified mouse cells and hope they can

be combined with the fibre to make a miniature artificial pancreas. They plan to test such a device in rodents later this year.

The researchers emphasize, however, that the mimicry is not yet perfect. The modified mouse cells secrete insulin at glucose levels well below the usual threshold. The researchers are confident they can dampen the modified cell's sensitivity by curbing the reactivity of an enzyme that metabolizes glucose after it has been shunted into the cell.

Unger suggests that even engineered cells that are too sensitive to glucose may be of benefit to people with severe, insulin-dependent diabetes. The insulin provided by such cells, he says, could help to make the disease manageable through diet.

Some critics of the approach warn that protecting the engineered cells from being ravaged by the recipient's immune system could yet prove a decisive stumbling block. The hollow fibre in which the researchers intend to package the cells is designed to keep out immune cells and antibodies while giving free passage to relatively small molecules such as glucose and insulin. Terry Atkins of Aston University warns that the fibre may not exclude small proteins such as interleukins which can attack foreign cells. (Source: *New Scientist*, 25 January 1992)

How drug defeats malaria parasite

The possibility of a new generation of malaria drugs has moved closer with a discovery by researchers in the United States. Scientists in Manhasset, New York, believe they have finally understood the mechanism of *Chloroquine*, the principal antimalarial drug. This knowledge is the first step to "designing" alternative compounds capable of disabling the malaria parasite, say the researchers.

Chloroquine's power seems to lie in its ability to disrupt a defence mechanism of the parasite, *Plasmodium falciparum*. The parasite feeds on the oxygen-carrying compound haemoglobin which is found in red blood cells. One of the breakdown products of haemoglobin is haem, a red pigment which is highly toxic to the parasite. Normally, *P. falciparum* copes with the haem by turning it into a less harmful brown pigment called haemozoin.

Scientists at the Picower Institute for Medical Research in Manhasset, New York, have identified the enzyme the parasite uses to make haemozoin. They separated it from an extract of parasites which turned haem to haemozoin: the more enzyme, the more haem was converted.

When the researchers added *Chloroquine* to the extract, they found that it inhibited the formation of haemozoin at concentrations equivalent to those found in the blood cells of a person taking the drug. This suggests to them that *Chloroquine* interferes with the parasite's ability to break down the toxic haem. "A detailed understanding of the mechanism of action of haem polymerase (the enzyme) would open the possibility of the rational design of new classes of anti-malarial drugs", say the researchers. (Source: *New Scientist*, 11 January 1992)

Treatment for haemophilia B

The British Technology Group (BTG) has signed a second world-wide licensing agreement to a US company for rights to develop gene products for the genetic therapy of haemophilia. The agreement, which is the result of collaborative work between BTG Ltd. and its North American affiliate, British Technology Group USA Inc., enables Transkaryotic Therapies Inc. (TKT) to develop a treatment for haemophilia B using TKT's proprietary gene therapy technology with BTG's patented cloned human gene for Factor IX.

TKT's gene therapy technology offers the prospect of incorporating the cloned gene for Factor IX into the cells of haemophilia B sufferers, allowing the continuous *in situ* production of Factor IX. Current therapy involves the intravenous administration of Factor IX concentrates prepared from human blood donations, and as such can expose patients to the risk of blood-borne viruses, e.g. AIDS and hepatitis.

TKT's approach to the treatment of haemophilia B would involve a physician taking a tiny sample of a patient's skin and sending it to TKT's laboratories, where the cells would be genetically engineered to produce Factor IX.

The modified cells would then be returned to the physician for injection under the patient's skin. TKT anticipates that clinical trials in this area will begin in 1993.

Details from: British Technology Group Ltd., 101 Newington Causeway, London SE1 6BU or on 071 403 6666. Fax: 071 403 7586. (Source: *Biotechnology Bulletin*, April 1992)

New treatment for bacterial infections

A powerful natural antibiotic may hold the key to treating many serious bacterial infections. The agent, called bactericidal/permeability increasing protein, or BPI, exists naturally in human white blood cells. BPI is toxic only for gram-negative bacteria. New York University (NYU) scientists have used the cloned gene to produce larger quantities of BPI and derivative fragments that contain the active regions of the molecule. In laboratory experiments, a fragment of natural BPI when added to whole blood both killed bacteria and stopped the self-destructive host responses triggered by endotoxin, a poison released by gram-negative bacteria.

The research was conducted by NYU in collaboration with Xoma Corp. at its laboratories in Berkeley and Santa Monica, California. (Source: *BFE*, Vol. 9, No. 1-2, January/February 1992).

Type D retrovirus in humans detected

Onasco Biotechnologies, Inc. (Houston, Texas) has developed, patented and begun to market two diagnostic kits that detect the new type D retrovirus in blood. Type D retroviruses are the predominant cause of AIDS in monkeys, a disease similar to that seen in human AIDS patients. A type D retrovirus was first isolated in 1970 from the breast cancer of a monkey and has since been associated with other cancers in monkeys, in addition to causing simian AIDS.

Since the current methods of screening blood supply will not detect type D retroviruses, and now that it has been found in humans, Onasco Biotechnologies will pursue both FDA and USDA licensing of its diagnostic kits. The company is currently negotiating with a public company to provide the necessary funding to obtain USDA/FDA approval of the diagnostic kits. (Source: *Genetic Engineering News*, January 1992)

Strategic partnership to offer "genes to cGMP" service

Two biotechnology companies from Canada and the United States have formed an international strategic partnership to provide bioprocessing expertise to the pharmaceutical industry worldwide. This alliance will allow Allelix Biopharmaceuticals Inc. to generate additional contract revenues for further development of its manufacturing technology and capacity. Verax Corporation, which currently provides a complete line of bioreactors, products and contract manufacturing services for mammalian cells, will now add bacterial and fungal expression systems to its product offerings, through the alliance with Allelix.

Allelix Biopharmaceuticals, in support of its own drug development programmes, has developed significant expertise in bacterial and fungal expression systems and offers contract research services such as genetic engineering, process development and scale-up for the manufacture of pharmaceutical proteins. Verax Corporation has a complete "Genes to cGMP" programme, through which it offers its customers bioreactor systems for in-house manufacture as well as bioprocessing contract development and contract manufacture of clinical trial and toll quantities of products made in compliance with FDA standards for current Good Manufacturing Practice (cGMP). (Source: *News Release*, 11 May 1992)

Cholesterol enzyme manipulated to prevent heart disease

Researchers at Northeastern Ohio Universities College of Medicine in Rootstown are developing a transgenic animal model to study how manipulating a key enzyme involved in cholesterol catabolism may translate to the prevention of heart disease.

Dr. Ferenc Hutterer, professor and chairman of biochemistry and molecular pathology, and Dr. Y.L. Chiang first purified cholesterol 7-alpha-hydroxylase in 1983. Then Dr. Chiang went on to clone, sequence and express the enzyme. Now the team is investigating the potential prevention of atherosclerosis from the perspective of the cholesterol degradation pathway. Atherosclerosis results from the pathological thickening and hardening of arteries when cholesterol and other compounds deposit on the vessel wall. Ultimately, plaques block blood-flow and cause either haemorrhage or thrombus formation.

The American Heart Association (AHA) cites three major advances in heart disease research: the discovery of cell surface low density lipoprotein (LDL) receptors (which won Drs. Joseph Goldstein and Michael Brown the 1985 Nobel Prize); the Coronary Primary Prevention Trial, which demonstrated that lowering cholesterol levels reduces death from heart attack; and the development of cholesterol-lowering drugs.

Specifically, Drs. Hutterer and Chiang study the rate limiting 7-alpha-hydroxylase enzyme, which is critical in breaking down cholesterol to bile acids.

The scientists created transgenic mice that carry the rat gene for 7-alpha-hydroxylase and propose feeding these mice a cholesterol-rich diet to induce atherosclerosis. The model may help determine if the rat gene, expressing high levels of enzyme, can prevent the mouse from succumbing to disease. The research is just beginning, but the scientists are hopeful that the transgenic model will support the concept that atherosclerosis is preventable by augmenting cholesterol degradation.

Since 7-alpha-hydroxylase's activity is lower in humans than in rats, and because humans are susceptible to atherosclerosis and rats are not, Drs. Hutterer and Chiang chose the rat as a model to study the relationship between the disease and the enzyme. A rat gene coding for the enzyme was successfully transferred to a mouse. Although it is still too early to see if the gene is expressed in the transgenic animal, it is possible that the mouse can acquire disease resistance if provided with a key degradative enzyme.

The final test of the gene's efficacy is if the enzyme encoded in the rat gene actually confers disease protection on the mice fed a cholesterol diet.

The direct human application of this research is in gene therapy. Familial hypercholesterolaemia is a genetic disorder in which there is an unusually enhanced deposition of lipid compounds in the arteries, leading to extremely early onset of the disease. The disease is caused by a mutation in the LDL receptor and represents the human model that initially focused attention on the relationship between LDL and cholesterol metabolism. (Source: *Genetic Engineering News*, 15 March 1992)

Pilot project for the production of anti-malaria drugs

A research project to improve production methods of anti-malaria drugs, based on the herb *Artemisia annua* has been established by the Ministry of Health of Viet Nam, in cooperation with the University of Amsterdam (The Netherlands). Main activities under this project are:

- The improvement of agricultural practices to produce, store and process Artemisia;
- Improvement of extraction and fermentation processes to produce *artemisinin* (the active compound of Artemisia), and the manufacturing of pills;
- Clinical studies in order to determine the correct application and dose of the drug.

The pilot plant is expected to produce 250 kilogrammes of pure artemisinin annually. As a full ton of dried leaves is needed to produce about six kilogrammes of artemisinin, a total area of 40 hectares must be planted with the herb in order to supply the raw material input.

Funds for the project - about US$ 1.3 million for 1992-1994 - will be provided by the Directorate-General for International Cooperation (The Netherlands). It runs parallel to a project started by the World Health Organization, in cooperation with the pharmaceutical company ACF Chemie (The Netherlands), that also aims at the production of Artemisia-based drugs. Artemisinine is a promising drug for malaria treatment, without unwanted side-effects. It might substitute for the traditional anti-malaria drug quinine, also a plant-based drug, against which the malaria parasites have developed resistance. In China and Viet Nam, extracts of Artemisia have been used for centuries for the treatment of fever, dysentery, infections and malaria. (Source: *Biotechnology and Development Monitor*, No. 9, December 1991)

Another gene therapy first

On 4 June, researchers at the University of Michigan Medical Center marked another milestone on the road to human gene therapy by injecting a gene into a tumour of a 67-year-old woman with malignant melanoma - the first time a new gene has been put directly into a human patient instead of being transferred first into the patient's cells in laboratory culture. The trial also represents the first time that researchers have used a non-viral vector - in this case liposomes - to deliver the modified gene.

Twelve patients with malignant melanoma, all with life expectancies of less than a year, will participate in the trial. Researchers are administering varying doses of genes for a transplantation antigen that is supposed to stimulate an immune attack on the patients' tumours.

Meanwhile, another Michigan team, led by James Wilson, has begun a gene therapy trial for patients with a hereditary form of high blood cholesterol. Using the more standard approach, the researchers introduce a virus carrying the gene needed to lower cholesterol levels into the patients' liver cells, which have been removed surgically. The cells with the new gene are then injected back into a vein that leads to the liver, in hopes that this will correct the patient's defect. (Source: *Science*, Vol. 256, 19 June 1992, p. 1628)

Silk wastes yield hangover treatment

Animal experiments by Professor Kiyoshi Hirabayashi of the Faculty of Technology at the Tokyo University of Agriculture and Technology have shown that a silk protein can lower blood levels of alcohol and cholesterol. If clinical studies support the finding, the protein may be put to work in preventing hangover. The starting point for Hirabayashi's experiments was an interest in finding ways to use the large quantities of waste silk generated in silk-producing regions. He found that hydrochloric acid treatment of silk waste yields a protein consisting of 18 types of amino acid, including glycine, serine, tyrosine and alanine. In the experiments, Hirabayashi fed 24-hour-fasted rats with a powdered form of the protein, then gave them ethanol 40 minutes later. According to results, the mean blood alcohol level of the rats was 30 per cent lower than that of control rats given ethanol without the protein. In further experiments, Hirabayashi gave rats feed with a 5 per cent content of the protein for one month. Results showed that the mean blood

cholesterol level of the rats was about half that of control rats given the same feed without the protein. Hirabayashi says this result is probably due to the action of serine and glycine. Hirabayashi plans to continue with research on the protein with the ultimate aim of developing functional foods for preventing hangover and hyperlipaemia. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 January 1992)

Livestock applications

Bioreactors on the hoof

Despite technical advances, the production of therapeutic proteins for the treatment of blood clotting disorders remains a difficult and expensive procedure. At the Virginia Polytechnic Institute, researchers working with the American Red Cross (ARC) believe the answer is not so much in the laboratory, as in the farmyard.

The team is harvesting an anti-clotting agent, human Protein C (hPC), from the milk of transgenic pigs. The protein, which is vital to the regulation of blood clotting in the body, could be useful in the treatment of patients at risk from so-called secondary clotting following a heart attack, blood infection or major trauma. Protein derived from human plasma by the ARC is now in preliminary clinical trials.

Although some proteins can be produced *in vitro*, such methods have their limitations.

Recently researchers in Scotland met with similar success, producing therapeutic proteins in transgenic sheep.

It is reported that each transgenic pig is producing much higher levels of hPC than is found in human plasma, and once the transgenic animal is in hand, modern animal husbandry can do the rest. Genie, the group's original transgenic pig, has passed the gene for hPC on to four of her seven piglets, and her sister has passed the trait on to six of her nine progeny. In total, the group now has some 30 transgenic pigs.

The approach offers high yields, low operating costs and simple access to the product. The team has already developed a technique to separate hPC from other proteins in the pigs' milk. (Source: *Chemistry & Industry*, 20 April 1992)

Brussels BST decision blow for manufacturers

Manufacturers of bovine somatotropin (BST) experienced another reverse on 15 January when the European Commission agreed a report on BST that concludes that use of the growth hormone would run counter to its efforts to cut back farm production. The Commission accepts that BST has no discernible effect on humans who consume milk from treated cows, although daily injection of the hormone can cause swelling and mastitis. EC agricultural ministers had already decided to extend the ban on BST by a further two years. (Source: *Biotechnology Bulletin*, February 1992)

AAT from ewe's milk

Milk from a sheep called Tracy is the key to a £10 million deal between German pharmaceutical giant Bayer and Scottish biotechnology company Pharmaceutical Proteins. Tracy is a transgenic animal - when she was no more than an embryo, human DNA was implanted into her genetic material, with the result that her milk now contains in excess of 30 g/l of the protein alpha-1-antitrypsin (AAT).

Deficiency of AAT is the most common human genetic disorder, affecting one in 2,000 people. AAT is a major inhibitor of the elastase enzyme, which is responsible for protein degradation in the tissue renewal process. Doses of AAT required for therapy are high - around 4 g/week - and current methods of production, which involve extraction from human plasma, cannot keep up with the demand.

According to Pharmaceutical Proteins' marketing director, Martyn Breeze, this is the first large-scale commercial exploitation of a product manufactured in this way. A flock of around a thousand "Tracys" will be bred, a process that has started already with the birth of Tracy's lamb. The flock of "Tracys" will lead a pleasant life, kept separate from other animals to avoid the risk of infection and with absolutely no chance of ending up as lamb chops.

Breeze expects that the first Tracy-derived AAT will reach the market towards the end of the decade. Pharmaceutical Proteins claims to be able to produce several proteins by these techniques, and is currently working on producing the blood-clotting agent Factor IX. (Source: *Chemistry & Industry*, 2 March 1992)

Australian sheep let their hair down

Scientists in Australia can now "peel" sheep rather than shear them. The technique relies on an artificial epidermal growth factor created by researchers at the CSIRO. When injected, it interrupts hair growth. A month later, breaks appear in the wool fibre and the fleece can be pulled off whole in half the time it takes to shear a sheep. Scientists at the CSIRO say they intend to genetically engineer a sheep that secretes insect repellent from its hair follicles. The creature is needed to fend off the blowfly, a pest that costs Australian farmers A$ 200 million (£85 million) a year. As a spin-off, the sheep will also shed the world's first mothproof wool. (Source: *New Scientist*, 4 January 1992)

Vaccine protects monkeys from AIDS-like disease

A vaccine based on a fragment of virus has succeeded in protecting monkeys from an AIDS-like disease. The finding increases optimism that a similar type of vaccine could be developed against HIV in humans. Many macaque monkeys have already been protected from the simian immunodeficiency virus (SIV) that causes the equivalent of AIDS in these animals. But virtually all successful vaccines have been based on preparations of whole killed SIV. Many scientists think it would be unwise to use whole killed HIV in humans.

Shiu-Lok Hu and colleagues at the Bristol-Myers Squibb Pharmaceutical Research Institute in Seattle have published details of a different approach. They vaccinated four macaques, first with live vaccinia virus which had been genetically engineered to produce the envelope protein of SIV. After more than a year, the animals received a booster of the viral envelope protein alone.

A few weeks later, the monkeys were "challenged" with an intravenous dose of live SIV to see if the vaccine would protect them. Four control monkeys, which had not been vaccinated, were also challenged. Within two months, all four control animals began to produce antibodies to the virus, showing that they had become infected. However, all four immunized animals remain completely free of virus more than a year after the challenge. This suggests, say Hu and his colleagues, that the vaccine protected the monkeys completely.

Bristol-Myers Squibb is already producing a comparable vaccine, which is being tested for safety in humans. Genetically engineered vaccinia virus expressing HIV's envelope protein is given first, followed by the envelope protein alone. But no one has tested the vaccine in field trials to show whether it protects people.

The results come at a time when AIDS researchers have been debating the way that SIV vaccines work. Recent British findings surprised researchers because they suggest that proteins from the human cells in which the whole-virus vaccines were grown have played a part in protecting animals from infection. It seems unlikely that these proteins were involved in Hu's experiment, because only recombinant viral proteins have been used. Until there is more evidence, researchers believe that both viral and cellular proteins must be explored as targets for human vaccines. (Source: *New Scientist*, 1 February 1992)

ACTIP meeting in Rome results in position papers on research

A meeting of ACTIP (Animal Cell Technology Industrial Platform) took place in Rome on 11-12 November 1991, hosted by Ares Serono. The members of the platform are European companies that use mammalian cell cultures in various phases of pharmaco-toxicological research and biotechnology research and production.

The EC-T-project Animal Cell Technology was one of the reasons to establish ACTIP, since ACTIP members will provide the European Commission with guidelines regarding Community-funded research programmes. T-project coordinators informed the meeting on the progress of their respective projects. The projects are on the techniques for establishing high expression production systems for recombinant proteins; construction of artificial chromosomes; optimization and validation of virus-based linear vectors; control of recombinant-DNA protein glycosylation; improvement of production of bioactive proteins; construction of permanently transfected cells expressing steroid hormone receptors; transgenic antibodies and targeted inducible amplified homologous expression systems for quality products from animal cells in culture. In these EC-financed projects, which involve companies and universities, a considerable amount of knowledge and input are used for worthwhile, ambitious goals.

This part of the meeting was chaired by Dr. Jan Lupker of Sanofi Elf Bio Recherches, who outlined the purpose of establishing a common view on future lines of research. The discussion has been initiated by the European Commission with the intention of receiving from industries with activities in animal cell technology a statement as to which fields of research should be publicly funded in future community research programmes. A first discussion took place during the ACTIP meeting in Toulouse on 23 April 1991. During that discussion various topics were reviewed. The major conclusion was that industry does favour public funding of research on biological issues, since lack of fundamental biological know-how is the bottleneck for many applications of animal cell technology. A position paper, identifying future lines of research and approved by ACTIP member companies, has been sent to the European Commission, DG XII.

In addition, ACTIP drafted a position paper for a research programme on unconventional agents of spongiform encephalopathy (BSE). ACTIP suggests a programme on fundamental research and establishment and identification of the nature of the agent; and development of rapid, sensitive diagnostic tests.

(Approaches which can be developed are: Western blotting using PrP antiserum and cell culture-based systems using neuronal-type cell lines). This position paper has been sent to DG XII of the European Commission as well.

During 1992 ACTIP will publish a leaflet explaining the reason for its existence, its charter, the names of company members and its achievements.

For more information, please contact Dr. Helma Hermans, Secretary, ACTIP, p/o Scientific Writing & Consultancy, P.O. Box 23 161, 3001 KD Rotterdam, The Netherlands. (Source: *News Release*, 15 January 1992)

Super tilapia promises fish farming revolution

Researchers at Muñoz, Philippines, through generations of breeding, have developed a super strain of tilapia that could revolutionize fish farming throughout the developing world.

Scientists at the International Center for Living Aquatic Resources Management (ICLARM) are calling the small, plump freshwater fish an "aquatic chicken".

Roger Pullin, who directs aquaculture research at ICLARM, says "The tilapia has remarkable attributes as a food fish. It is delicious to eat, with no fine intramuscular bones. It is also easy to breed, cheap to feed, tolerant of wide ranges of temperature, salinity and water quality - and comparatively free from parasites and diseases."

Mr. Pullin and other experts at ICLARM have dedicated years to improving the tilapia through careful breeding. Their research has produced a hybrid tilapia that is larger and stronger than those farmed today in Asia. This tilapia is about to be introduced to Filipino fish farmers. ICLARM scientists expect it to herald a "blue revolution" in fish farming as important as the "green revolution" in food crops a quarter of a century ago.

This promise is based on the hybrid tilapia's ability to thrive under harsh conditions - which means that they can be farmed by poor families throughout the developing world.

Unlike other tilapias and tropical aquaculture fish, the hybrid tilapia prospers in flooded rice fields, vegetation-choked ponds, or brackish water. They reach full size in less than 75 days - a full 30 days ahead of other tilapia species. The short maturing time means larger sales for poor farmers. It also means that rice farmers can stock their fields with the hybrid tilapias - impossible with slow-growing fish because rice fields are flooded for only three months at a stretch. And unlike most other fish currently used in tropical aquaculture, the hybrid tilapias do not require hormones or complex hatchery equipment to breed.

ICLARM scientists expect the new tilapia's plus points to make a significant impact on tropical fish farming. In South-East Asia, where tilapias are already a major farm fish, they conservatively expect a 40 per cent jump in tilapia production once the hybrid is widely distributed to farmers. Tilapia farmers will at minimum net a 30 per cent increase in income. The hybrid may lead to large increases in Africa, where tilapias are the most important aquaculture fish. (Extracted from *World Development*, 1 January 1992)

Agricultural applications

Modern agriculture versus biological diversity

Modern agriculture reduces biological diversity and stability by using just a few varieties of animals and crops, rather than locally adapted varieties. Even the modern "high-yield" crop varieties do not always produce high yields. They often need irrigation, pesticides, fertilizers and good soil. If any of these ingredients is missing, modern varieties may produce less than the variety that was displaced. And if the local varieties have become extinct in the meantime, farmers will be worse off than ever if the modern varieties run into some problem for which they are not adapted.

In Kenya, the Kenya Energy and Environment Organisation is trying to help small farmers by focusing on indigenous vegetables. Dek, for example, has three times as much vitamin A and protein and five times as much vitamin C as an introduced vegetable such as cabbage. Dek grows wild, and is also cultivated in small gardens, along with 20 other local vegetables. Farmers need such diversity to prevent catastrophe, should one or more crops fail. But some governments will provide aid to farmers only if they switch to modern varieties and use insecticides and fertilizers. The University of the Philippines has in just three years identified a number of local crop varieties that are better than the "high-yield" varieties, but 80 per cent of farmers in the Philippines are landless tenants, who have no stake in preserving the land. Roger Mpande of ZERO environmental consultants in Zimbabwe says governments are too often the problem. The Government still subsidizes maize, although it is ill-suited to the land. Ethiopia's Plant Genetic Resources Centre has paid farmers to grow local varieties alongside modern varieties. The local varieties won. Even in the EC, there are laws against selling seeds of any vegetable variety not on the approved list. As a result, many useful varieties have been lost. (Extracted from *New Scientist*, 9 May 1992)

Awesome secret of the Indiana banana

The Indiana banana - in fact a species of pawpaw and not a banana - contains a chemical many times more toxic to tumour cells than either the taxol that comes

from yew or conventional anti-cancer drugs. The tree grows wild all over the eastern states of the United States.

Conventional anti-cancer drugs, such as adriamycin and cisplatin, interfere with the DNA in tumour cells, preventing them from multiplying. The pawpaw's family, the *Annonaceae*, is a rich source of a group of chemicals called acetogenins. These are forms of fatty acid lactones which interfere with the electron transport system in the mitochondria of cells. The major active ingredient of the Indiana banana is asimicin. Tests of the extract on mice with cancers of the ovary were very successful.

Researchers from Purdue University (Indiana, USA) have looked at 40 other species of the *Annonaceae*. They found a single specimen in Miami, chopped off three of its five branches and screened an extract of the tree. They isolated 11 active compounds. One compound, called bullatacin, was a billion times as powerful as adriamycin and 300 times as potent as taxol. Small branches and twigs yielded the highest concentrations.

A crude extract from pawpaw twigs kills all manner of pests, from aphids to bean beetles, spider mites and nematodes.

The group has identified dozens of potentially useful compounds from plants. The team's success is due to their cheap and easy method of screening plant extracts. The group tests the activity of an extract by adding it to a solution of sea water containing brine shrimps - and count how many die. Promising compounds go on to more rigorous tests in cell cultures and animals.

The test for anti-cancer properties is just as simple: it consists of inducing crown gall tumours in small discs of potato and seeing whether the chemical inhibits tumour growth. If it does, the substance will probably have the same effect on human tumours. (Source: *New Scientist*, 15 February 1992)

Shiitake mushrooms promote rice growth

Results of experiments commissioned by Noda Shokuhin Kogyo Co., Ltd. of Chiba Prefecture (Japan) show that a shiitake mushroom (*Cortinellus shiitake*) extract promotes the growth of rice seedlings. Noda Shokuhin is marketing the extract for antiviral treatment of orchid plants. The extract is also a component of the company's health beverage "C-Kin Drink".

The company enlisted the expertise of the Yamagata Prefecture Agriculture Experiment Station (YPAES) to run experiments in a bid to develop further applications of the extract. The shiitake mushroom extract contains growth hormone and glycoproteins. YPAES researchers found that treating rice seedlings with the extract prior to field planting led to more extensive root development and an increase in the dry weights of stems and leaves. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1992)

Fast-growing reeds could fuel Europe's future

Trials are just beginning in Britain of a giant reed which may have a higher yield as a biomass fuel than any other crop. In Germany, research with the crop is further advanced and more than 130 hectares of trial plots are growing this year. Farmers and industrial companies are examining its potential as a fuel and as a raw material for making paper and chipboard.

The reed is a perennial called *Miscanthus senensis*, native to northern China and Japan. German researchers claim that it can yield 30 tons of dry matter per hectare per year.

Miscanthus meets many of the requirements of an ideal biomass crop: it is dry when harvested, burns cleanly and can be harvested annually. *Miscanthus* also has a different metabolic pathway for photosynthesis. It is more characteristic of subtropical plants, thrives in high light and high temperature conditions and uses water more efficiently.

As with any biomass fuel, any carbon dioxide produced when the reed is burnt was extracted from the air when it grew, so producing and burning it does not add carbon dioxide to the atmosphere. There are other environmental benefits as well. "It offers high production on minimal chemical inputs", explains Manfred Dambroth of the Federal Research Institute for Plant Breeding and Crop Husbandry in Brunswick. "No pesticide sprays are required and the plants' rhizomatous root system has proved to absorb fertilizers efficiently, so helping prevent seepage of nitrate into groundwater."

In Germany, some farmers are pressing ahead with production. A group of Bavarian growers has signed contracts to supply *Miscanthus* to a local crop-drying cooperative. Twenty hectares of *Miscanthus* have been planted. The plan is for the crop drier to change over from heating oil to 80 per cent home-grown fuel by 1995.

In Dresden, a paper manufacturer is testing locally grown *Miscanthus* as a cellulose source instead of timber. Its tests have shown cellulose from *Miscanthus* to be just as good as that from timber and better than that from any other annual farm crop.

VEBA, an oil and chemicals company near Gelsenkirchen in the Ruhr, has planted over 30 hectares of *Miscanthus* plots this year. The crop is used to produce hydrogen gas which in turn is used in the refining process for crude oil.

The most obvious users for *Miscanthus* would be power stations. "Systems must be created: with farmers supplying the *Miscanthus* on contract to small community central heating and power stations, for instance", says Dambroth. "Machinery must also be developed for harvesting and packing the *Miscanthus* into tight bales for efficient transport and handling".

One problem with the crop as a power source is that it is all harvested at one time in the year. It would be suitable for integrated power stations which at different times of year could burn *Miscanthus*, wood chips from coppicing, straw or even domestic waste. (Source: *New Scientist*, 9 May 1992)

Biotechnology threatens to extend pesticide era

The first major products of agricultural biotechnology, instead of ending dependence on toxic agricultural chemicals, threaten to further entrench and extend the pesticide era, according to a report, "Biotechnology's Bitter Harvest", prepared by the Biotechnology Working Group in the USA.

The report says many biotechnology companies are now using genetic engineering to modify major crops to make them tolerate lethal doses of herbicides. The widespread use of herbicide-tolerant crops will perpetuate and even broaden the use of herbicides.

Herbicides are chemicals intended to be poisonous to weeds, but they have been found to contaminate food and groundwater, and available studies link them with birth defects, skin diseases and cancer.

The report has also warned that widespread adoption of herbicide tolerant crops in the third world could erode the genetic diversity of crop and wild plants, and exacerbate pesticide-caused human and environmental problems.

The working group has representatives from public interest organizations, an agricultural agency and citizen activists working on biotechnology-related issues in the environment, agriculture and public health fields. (Source: *Chemical Business*, 5 January - 4 February 1992)

"Friendly" insecticide found

Natural insecticides that kill aphids have been found in fungus-infected tall fescue grass. Chemists Richard J. Petroski and Richard G. Powell of USDA's Agricultural Research Service (ARS) say the compounds, called "N-acetyl loline" derivatives or lolines, offer potential as environmentally-friendly insecticides against aphids and other pests in gardens and houseplants.

Working at the national centre for agricultural utilization research at Peoria, Illinois, the scientists extracted the compounds from tall fescue infected with the fungus *Acremonium coenophialum*. ARS collaborators at the northern grain insects research laboratory, Brookings, South Dakota, then sprayed solutions of the compounds on barley plants infested with greenburg aphids. The greenhouse tests showed five of the compounds were nearly as effective at killing the aphids as nicotine sulphate, a home and garden insecticide popular before modern synthetic insecticides became available.

As an example, a 0.05 per cent solution of N-acetyl loline in water sprayed on plants killed 50 per cent of the aphids.

Lolines would be fairly expensive to extract from plants because they generally are found in low concentrations. A biotechnology technique may make it possible to produce lolines at a lower cost in the future. (Source: *Chemical Marketing Reporter*, 30 December 1991)

Wine and roses

The French luxury goods research group LVMH (Moët Hennessy Louis Vuitton) has successfully transferred genes into a grapevine root stock and for a variety of rose bush.

The viticulture laboratory at Moët & Chandon, one of the biggest champagne houses, has transplanted a ma er gene into a root stock called 41 B and expects "within months" to grow the vine with a gene that will protect it from one of its most devastating viral diseases.

The root stock 41 B, the second commonest root stock in vineyards around the world, is used extensively in Champagne and the cognac-producing region of Charente.

The central research laboratory of LVMH has also grown a variety of rose with a gene that should increase the number of blooms. This transgenic rose bush is just beginning to produce flowers and LVMH expects to know within six months to a year whether it is really more productive. (Source: *New Scientist*, 4 April 1992)

Will altered crop genes run wild in the country?

Foreign genes introduced into crops pollinated by insects may quickly spread into wild varieties, according to biologists in the United States. Pollen carrying the genes could fertilize wild varieties of crops a kilometre away or more.

These findings increase fears that weeds will crossbreed with genetically engineered crops, inheriting traits which make them better able to survive drought, frost or pests. Norman Ellstrand of the University of California at Riverside and his colleagues tracked the spread of genetic material from a central plot of cultivated radishes called "Round Whites" to wild radishes (*Raphanus sativus*) planted at several sites up to a kilometre away from the central plot.

By examining seeds from the wild radishes, the researchers were able to track the spread of a gene from the Round White which shows up when seeds are stained. Plants producing seeds that contained the gene must have been fertilized with pollen from the Round Whites.

Ellstrand was not surprised to find that the closest wild radishes - those only a metre from the plot of Round Whites - were the ones most heavily pollinated. Between 14 and 100 per cent of the seeds harvested from these plants had acquired the foreign gene.

The rate of gene transfer decreased sharply with distance. But, surprisingly, some seeds at the one-kilometre margin of the experiment contained the gene.

Ellstrand estimates that up to a third of all crop varieties are pollinated by insects, and should be treated with extra caution in gene transfer experiments.

One safety-first approach, he suggests, would be to modify engineered plants so that they are male-sterile and produce either no pollen or pollen that is inactive. (Source: *New Scientist*, 21 March 1992)

Gene transplant gives apricots a riper future

For the first time, researchers have successfully transferred a beneficial gene into tree cells and grown saplings carrying the gene. If the transplanted DNA protects the young trees against disease, as the researchers hope, the technique could have immense economic consequences across eastern and southern Europe.

Margit Laimer and her team, from the Institute of Applied Microbiology at the Agriculture and Forestry University in Vienna, targeted Sharka disease, which is caused by the plum pox virus (PPV), the most damaging pathogen of stone-fruit trees. Among the most commercially valuable victims of the pox are species of *Prunus* - apricots, peaches and plums. The disease deforms the fruit and makes them ripen unevenly. Sharka is widespread in eastern Europe and Mediterranean countries. The virus is carried by aphids. There is no cure and an infected tree is of no use within two to three years, so it should really be cut down as soon as the symptoms are detected.

Laimer's team worked with cells from the apricot, *Prunus armeniaca*. Into these they added one of the genes that codes for the virus' protein coat. Earlier work with herbaceous plants showed that this gene provides resistance to the virus, rather like a vaccine, although the mechanism is still not properly understood.

To transfer the protein coat gene, Laimer and colleagues used the bacterium *Agrobacterium tumefaciens*, which naturally infects plant cells. They spliced the gene into a circular piece of DNA, or plasmid, within the bacterium. Only this DNA is integrated into the plant genome, says Laimer.

She believes that genetic engineering to protect woody species against a range of infections will soon be commonplace.

While many herbaceous crop species such as alfalfa, potato, tobacco and tomato have had "foreign" genes introduced into them, work on woody plants has not gone beyond testing the feasibility of methods for transferring genes into cells.

Details of the team's research will be published in *Plant Cell Reports*. (Source: *New Scientist*, 14 March 1992)

The wonders of the neem tree

An upcoming report from the National Research Council (NRC) will tout the wonders of the neem, a tropical tree whose seed kernels can be used to produce pesticides, medicines, even a potent spermicide - which is why the report's authors believe the neem could boost the economies of developing countries. The NRC staff has been investigating neem for a couple of years.

Pesticides derived from the tree, which is sometimes called the margosa and is native to India and Burma, have shown "remarkable effectiveness" against more than 200 species of insects, including mosquitoes and the desert locust. Other neem compounds fight tooth decay, viruses and a variety of bacteria, says David Unander, a plant breeder at the Fox Chase Cancer Center in Philadelphia and an NRC panel member.

The biologically active, polar chemicals can be extracted using technology already available to villagers in developing countries.

Neem backers also claim that their wonder tree might make a dent in other global problems. The fast-growing tree is already being planted in deforested areas of Haiti and sub-Saharan Africa. Neem oil also appears to be a powerful spermicide that could help reduce overpopulation. (Extracted from *Science*, Vol. 255, 17 January 1992, p. 275)

Grain yields tumble in greenhouse world

Dried-up fields and empty grain stores are likely to become semi-permanent features across much of the third world by the middle of the next century, according to an analysis of the likely impact of global warming. The crippling drought in Southern Africa this year provides a graphic foretaste of things to come.

The new study suggests a decline of between 10 and 15 per cent in grain yields in Africa, tropical Latin America and much of India and South-East Asia, as well as a substantial increase in famine. Its author is Martin Parry, director of the Environmental Change Unit at the University of Oxford and a leading analyst of the agricultural implications of the greenhouse effect.

Most of the world's important food crops, including wheat, rice, soya beans, sorghum and millet, will be hit, says Parry. His study assesses the implications of regional changes in climate predicted by computer models run by Britain's Meteorological Office and NASA's Goddard Institute of Space Studies (GISS) in the United States. The models assume a doubling of carbon dioxide concentrations in the atmosphere.

The two models differ over what might happen to crops in the richer northern countries. While the GISS model suggests that crop yields could increase in places as hotter summers become more frequent in the temperate regions, the Met Office model suggests abandoned fields and declining harvests, especially in North America.

This finding contradicts recent studies presented to the White House by American agriculturalists, which suggest that US agriculture will be largely immune to damage from global warming.

Declining yields in North America could have a world-wide impact, says Parry. "Negative yield changes in North America would substantially raise grain prices worldwide", he said in Oxford, before leaving for the US to deliver his findings.

Parry is the author of a series of case studies on agriculture and the greenhouse effect, published two years ago by the International Institute for Applied Systems Analysis (IIASA). Since then he has become increasingly pessimistic: "The change is much more negative than I had previously guessed".

Parry's new findings follow a year's work for the UN's Intergovernmental Panel on Climate Change. He stresses that they are not firm predictions, but are "sensitivity studies". They show the vulnerability of the world's farms to climate change. The projections, he says, build in the ability of farming people to adapt to climate change by changing crops or farming methods.

To prevent hunger, the world must make breakthroughs in biotechnology, he says, creating new crops adapted to hotter climates and drier soils. There will also be a need for "major, and I mean very major, irrigation and land reclamation schemes". But, he adds, "the best way of adapting would be to reduce population growth".

Parry's studies also suggest that there will be increased demand for water to irrigate fields in most of Europe. (Source: *New Scientist*, 18 April 1992)

Researchers ask for help to save key biopesticide

Agricultural researchers want the United States Government and industry to adopt a more careful use of the biopesticide *bacillus thuringiensis* (Bt) to reduce the chances that crop pests will develop resistance to its toxicity.

In a report from the US Department of Agriculture (USDA), government and academic researchers call for precautions and potential regulations that would prevent insects from becoming immune to Bt. At stake is the viability of one of the most promising biopesticides ever developed.

Forty years ago Bt was just a bacteria found in ordinary dirt. Then, when researchers discovered that Bt produces toxic proteins that kill dozens of common insects (but are harmless to humans), it became a biopesticide to spray on crops. Over the last decade, however, as researchers discovered how to engineer the DNA of many plants so that they can express Bt toxins themselves, the simple bacteria has triggered a multi-million dollar research effort in agricultural industry to produce self-protecting crops that do not require chemicals to fight off pests.

Now, on the verge of a revolution in agriculture, researchers are afraid that Bt's miracles could be coming to an end. Several US teams have managed to create Bt-resistant pests in the laboratory in as little as 12 insect generations. And if it can be done in the laboratory, it can eventually happen in the real world, they argue, especially when Bt-engineered plants reach widespread use.

Insects that are susceptible to Bt toxins are "a genetic resource" says Mark Whalon of the Michigan State University.

Last month, at a meeting sponsored by the USDA, government and academic researchers hammered out a strategy they hope can save Bt. Their report is expected to contain 124 suggestions for research on Bt toxicity, monitoring programmes and precautions to avoid insect resistance.

Chief among the recommendations is an independent national advisory body that would serve as

a Bt guardian. Reporting to the USDA, the group would recommend research on the biopesticide and monitor its use. Farmers will have to be careful not to rely on Bt alone, says Whalon, one of the authors of the report. Instead, he says, they should try to "keep the insects off balance" by mixing Bt with some traditional chemical pesticides, natural predators and other biotoxins.

Researchers also hope to encourage farmers to plant non-Bt plants alongside their engineered cousins that will serve as a haven (and genetic reservoir) for Bt-susceptible pests. Varying the percentage of Bt-expressing plants from zero to 50 per cent in a single field should also help to lower the percentage of pests with resistance by allowing susceptible insects (which usually have some other reproductive advantage over resistant insects) to recover and drive out the competition. (Source: *Nature*, Vol. 355, 20 February 1992)

Programme aids developing world

A US-based centre hopes to help developing countries take advantage of advances in agricultural biotechnology with the support of private companies and foundations that want to stimulate that technology.

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) announced its home for the next five years would be at Cornell University in Ithaca, New York. ISAAA intends to help farmers increase yields and reduce their dependence on pesticides in a handful of countries that are best prepared to take advantage of the new technologies.

At present those farmers lack the money, technical skills and infrastructure to make use of this technology, which is becoming increasingly proprietary. What ISAAA hopes to do is tap into that extensive knowledge, now held mostly by private industry, and transfer it to developing countries without disrupting the way agriculture is carried out in those countries.

Previous efforts to transfer technology to developing countries have lacked sufficient people, technology and money in those countries. The models also have relied on laboratory-based initiatives between universities rather than people on the front lines. In contrast, ISAAA will invest not in laboratory facilities but rather in transferring tested technologies to those who can use them.

ISAAA has raised more than $4 million in almost 10 months of operation, but David Altman, ISAAA's president and executive director, says it will need almost three times that amount to be fully operational. ISAAA also has found financial backers for two projects in Mexico and Taiwan.

The service plans to have branches around the world to help its staff learn about proprietary technology in those regions. In addition to its AmeriCenter at Cornell University, Altman hopes by next year to open a EuroCenter in Norwich, United Kingdom. Plans also are afoot for an AsiaCenter in Japan, as well as locating staff in Africa, Asia and Latin America.

ISAAA will concentrate on 10 target countries within Asia, Latin America and the Middle East/Africa - places that ISAAA feels have both the capability and political will to implement agricultural programmes in biotechnology, and where the chances for success are greatest. It will also confine itself to applications that have been tested in industrialized countries and where field testing in the developing country can begin within five years.

Initially, ISAAA will focus on plant biotechnology applications in the areas of tissue culture, diagnostics and transgenic plants. Altman says that he favours projects that help poorer farmers to grow non-commercial crops, in particular vegetatively propagated and open-pollinated fruit and vegetables. Other projects will focus on forestry and the use of micropropagation techniques to develop tropical tree species that will contribute to biodiversity and which are difficult to propagate through seed. (Source: *Nature*, Vol. 356, 30 April 1992)

Making bamboo bloom: a revolution in a test tube

Indian scientists have tamed the "high emperor of all the grasses".

In a remarkable and still mysterious breakthrough, they have succeeded in making three species of bamboo bloom and produce fertile seeds in the laboratory. In doing so, they have for the first time put this globally important grass within reach of plant breeders and biotechnologists for selectively breeding and hybridizing newer strains.

Further refinements to their research could lead "to the introduction of a breeding programme to improve bamboo and the production of perennial seeds for bamboo, as well as to a better understanding of the physiology underlying flowering behaviour in bamboo", the scientists said.

Bamboo, which flowers only once in its lifetime and dies at the end of its first fruiting season, presented a unique problem. The seeds that are essential for mixing genes are produced only after flowering, and it takes a plant anywhere from 12 to 120 years to flower. R.S. Nadgauda, V.A. Parasharami and A.F. Mascarenhas of the plant tissue culture group of India's

National Chemical Laboratory in Pune took tiny cuttings from bamboo seedlings belonging to the species *Bambusa arundinacea, Dendrocalamus brandisii* and *Dendrocalamus strictus* and grew them in a special nutrient medium containing a cocktail of chemicals, including the plant hormone cytokinin, in coconut milk. Instead of waiting the normal 30 years to flower, these plants gave flowers and fertile seeds in the unbelievably short time of three months. No one, not even the researchers, is entirely sure why this happened, but it did - again and again as they repeated the experiment.

Although they are not yet sure, scientists suspect cytokinin is mainly responsible for promoting early flowering.

More than 500 species of bamboo grow all over the world, with their greatest concentration in Asia in the tropical monsoon forests of India, China, Japan and Korea. Bamboo can grow at an amazingly fast rate of four centimetres an hour and soar to heights of 40 metres in some species. A growth of up to 25 metres can occur in just a couple of weeks after the monsoon. Japan's most common bamboo grew a record 1.5 metres in just 24 hours.

Bamboo seems to possess a perfectly synchronized biological clock, which ensures that all members of the same origin, whichever part of the world they happen to be in, flower, seed and die at the same time. There have been reports of bamboo from India and China flowering in English gardens in clockwork precision with their relatives in Asia. After flowering, bamboo sets seeds and immediately dies. Huge expanses of bamboo forests disappear after a flowering season, leaving behind an apocalyptic scene of rotting culms, raging fires and a knee-deep carpet of seeds to be gorged on by hordes of rats and wild animals.

The ability to make bamboo bloom at man's will has opened up exciting possibilities for plant geneticists. They can now tailor hybrids to grow still faster, resist diseases and yield a stronger, more versatile wood. Mixing and matching hundreds of characteristics, biotechnologists will be able to obtain hybrids suitable for specialized applications. But perhaps the most important gain from this technology is that, for the first time, we can hope to have a perennial source of bamboo seed.

Researchers at Pune are now improving their techniques to determine the most favourable sets of environmental and nutritional conditions for flower induction and seed formation *in vitro*. Their experiments should lead to a better understanding of the precise nature of physiological and molecular events that dictate flowering patterns in bamboo. They should also shed new light on exactly how cytokinins speed up the biological clock in plants to promote early flowering. They may one day be able to answer the question of what makes members of the same species flower, set seed and die in unison, even after being burnt or savagely cut back anywhere on the planet. (Source: *Ceres*, January/February 1992)

Chemical applications

New materials are cropping up

With a fetish for natural fibres, James Bolton at the University of Wales, Bangor, is determined to demonstrate the "untapped potential" he sees in plants. For him, wood, flax, sisal, hemp, coir - and even straw - are the materials of the future.

The Bangor team is one of many European research groups now challenging the foundations of the chemical industry by doing away with the conventional petroleum feedstocks. Their work - perhaps even offering European farmers an alternative to overproduction of food - could bring a new range of materials made from crops.

Exploiting the inherent strength of natural fibres, Bolton's research team has developed some novel chemistry, which allows the fibres to be used in composites alongside petrochemical plastics. By using plant fibres in place of glass in reinforced plastics, Bolton can produce a range of strong, light-weight materials.

The key to Bolton's work is the modification of the fibres' surface chemistry to make the materials water-resistant. The patented process reacts the surface hydroxyl groups with difunctional reagents, such as anhydrides and isocyanates. The second functionality is then used to give good bonding properties with other fibres, or with the matrix adhesives used in many composite materials.

The natural fibres can be configured in a random matting, or woven together. After chemical treatment, the fibres form cross-links between one another and with the applied matrix adhesive. The resulting composite is "certainly in the same league as glass-reinforced products", Bolton says.

Through his BioComposites Centre, set up in 1989, Bolton and his 27 staff have attracted enough industrial interest for an investment of £1 million in a pilot facility aimed at developing the composites technology. The centre is mid-way through a three-year LINK research project - with Hoechst, Ciba-Geigy, and paper and composite board makers - aimed at refining the process.

Another research group, led by Fiat, is working on a fibre-and-plastic composites project under the European Community's ECLAIR research programme (European Collaborative Linkage of Agriculture and

Industry through Research). The researchers hope to develop a blend suitable for car body production.

As well as composite materials, however, the Bangor researchers are studying the use of natural fibres in pollution clean-up. Straw, chemically modified to resist water, can pick up more than 30 times its own weight of oil, Bolton says. A company is currently looking at developing the technology for use on garage floor spills. Bolton postulates systems such as large, floating booms for use on oil spills at sea.

By further modification of the fibres' surface chemistry, the centre has also developed systems for picking up heavy metal pollutants. With acid groups attached to the fibre surface, coir (coconut husk) can be made to pick up at least 1 per cent by weight of copper. The material would cost only £500/t, compared with more than £3,000/t for the conventional ion-exchange resins used in pollution clean-ups. After use, the material can be burned, leaving copper oxide as a residue.

In addition to research on composites, several groups are now working on plastics derived directly from agricultural products.

Beyond the green appeal of biodegradable plastics, the material's principal markets are therefore seen as niche areas in medicine and agriculture - particularly slow-release technologies.

ICI's research is continuing through an ECLAIR project aimed at improving the understanding of the biochemical pathways and the enzymes involved in producing Biopol - which is a polyhydroxyalkanoate (PHA).

New pathways for synthesizing PHA copolymers have been discovered, and ICI hopes to clone the genes controlling these pathways and transfer them into *Alcaligenes eutrophus*, the bacterium used to make Biopol. The partners - including the Georg-August Universat in Denmark, Rijksuniversiteit, Gent, and the University of Hull - are also studying the mechanisms for biodegradation.

Unilever's Unichema International business is also looking at ways of synthesizing polymers from plant oils. At its Colworth laboratory in Bedfordshire, the company is working with some mutant strains of Candida yeast which can synthesize a range of novel monomers.

The chemicals obtained are mostly activated fatty acids, such as dicarboxylic acids, hydroxy fatty acids and hydroxydicarboxylic acids. The yeast lives naturally on oils, and Unilever is looking at rape-seed, palm, coconut and sunflower as feedstocks - "whatever is available and cheap", according to John Casey of the speciality chemicals division.

Another polymers-from-oils project has recently started up at Mid-Kent College and London's South Bank Polytechnic. Led by Brian Keene at Mid-Kent, the researchers are developing ways of making polyurethanes from chemically modified rape-seed oils.

The specialized polyurethanes are used in the electrical industry in transmission equipment. "Tonnage" quantities of castor oil are currently used by Keene's industrial partner - a large electrical and electronics firm - to make the polyurethanes. He says that the alternative route from the cheaper rape-seed oil is showing "considerable promise".

In Bangor, meanwhile, Bolton has also been dabbling in plastics technology. The centre has made a rudimentary cling-film from soluble polysaccharides in straw and from food-grade celluloses.

The thermoplastic material is self-plasticizing, so that no additives are needed, and the much-publicized problems of migration of plasticizers into fatty foods are avoided. Since some of the starting materials are celluloses used as fillers in foods, Bolton believes that only limited toxicity testing would be required to launch the product for food-contact uses. The centre is applying for a patent on its cling-film.

Wax-like materials made from food-grade cellulose would be some four times more expensive than petrochemical waxes, Bolton believes. But they could be made in larger volumes and compete in areas like foods, cosmetics and pharmaceutical delivery systems.

Preliminary results indicate that the waxes and plastics could be made from polysaccharides from straw; the materials could eventually replace polyethylene and polypropylene in some applications. "They might just come in slightly cheaper", Bolton claims.

To refine the technology for extracting cellulose, Bolton is currently putting together a LINK research project on straw fractionation. While there are many projects under way on crop fractionation, most of these are focusing on mechanical separation of the various crop components. Bolton is interested in fractionation at the cellular level, to produce large volumes of cellulose feedstocks.

Mechanical crop fractionation could provide an economic supply of straw pulp for the paper industry. The Silsoe Research Institute in Bedford, UK, is working on an ECLAIR project on straw "slicing". The machine being constructed will be used to supply a pilot straw pulping plant.

Silsoe is also working on a large ECLAIR project led by Bioraf in Denmark, and involving the Danish biotechnology firm, Novo Nordisk. The pilot "biorefinery" under construction will integrate crop processing under a single roof.

The researchers are looking at enzymatic extraction of rape-seed oil and the recovery of proteins and starch. The plant will also mechanically fractionate crops and produce flours and feed ingredients. A key component of the project is an assessment to see if large-scale European biorefineries would make economic sense. (Source: *Chemistry & Industry*, 20 April 1992)

DNA fingerprinting

The current procedure for comparing DNA fingerprints is "fundamentally sound" and should be considered reliable when done properly, according to a report released by the National Academy of Sciences' National Research Council (NRC). But while the report should make the controversial technique more acceptable in the courtroom, it is not expected to end debate about its use.

The report* suggests additional studies and measures to improve the standards of laboratories that carry out the tests and to strengthen the statistical basis for making comparisons of DNA samples. The NRC panel recommended that researchers take DNA samples from 100 people in each of 15 to 20 ethnic groups and that they maintain a database of the blood samples to reduce the possibility that ethnic subgroups in populations can distort the chances of finding random matches. It also recommended that the US Department of Health and Human Services establish an accreditation programme to check the quality of forensic laboratories.

None of these suggestions are contentious. But its chapter on population genetics seems likely to renew debate over whether the technique is a good way to identify someone conclusively.

The US Federal Bureau of Investigation (FBI) and many prosecutors have used a "multiplication rule" to determine the odds of random matches. Using that technique, a laboratory comparing DNA samples at several locations on the chromosome would multiply together the known frequency of each DNA site in available databases. Critics, however, argue that this technique discounts the possibility that the patterns may be related and inherited together. But in the absence of extensive databases on ethnic subpopulations, researchers do not know which patterns are typically inherited together. (Source: *Nature*, Vol. 356, 16 April 1992)

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* *DNA Technology in Forensic Science*, National Research Council, 1992.

Energy and environmental applications

Battelle reports work on bioreclamation

Bioreclamation technology, which involves the biological treatment of contaminated soil and water, is an important research focus at Battelle. According to two Battelle scientists, Karl Nehring and Bob Olfenbuttel, the technology "involves three approaches: enhancing oxygen delivery to microorganisms, especially in the promising area of "bioventing"; enhancing the amount or kinds of nutrients available to microorganisms; or enhancing the amount, kind or characteristics of the microorganisms themselves". Battelle is improving techniques - such as advanced computer modelling and rigorously engineered systems - to enhance oxygen delivery and promote biodegradation. The Institute is currently using bioventing, a significant trend in enhanced oxygen delivery, in environmental settings from the frozen tundra of Eielson Air Force Base in Alaska to the desert conditions of Fallon Naval Air Station in Nevada.

In another project, Battelle enhanced available nutrients and used other bioventing techniques to significantly reduce volatilization rates. This allowed the direct discharge of vent gas into the atmosphere without off-gas treatment. Microbial strain development has implications for all approaches to bioreclamation and, with the advent of increasingly powerful genetic engineering techniques, will be a key bioreclamation trend. Battelle scientists are searching for more effective strains of microorganisms, such as methanotrophs, which use methane as their primary food source, to degrade both specific compounds and combinations of compounds unamenable to treatment by existing bioreclamation technologies. Details from: Battelle Institute Ltd., 15 Hanover Square, London W1R 9AJ, or on 071-493 0184. (Source: *Biotechnology Bulletin*, March 1992)

Biotechnology's answer to emissions

Celgene (Warren, New Jersey) says it has successfully demonstrated its biological treatment technology to reduce methylene chloride emissions at GE Plastics' Mount Vernon, Indiana plant. It is the first such treatment process to be installed on-line as part of a pollution prevention effort, according to Celgene. GE Plastics uses methylene chloride to make its engineering thermoplastic [illegible]. The Celgene process takes advant[illegible] organisms in a bioreactor to degrade th[illegible]oride in a liquid effluent, producing wate[illegible], carbon dioxide and - after neutralization - salt. GE Plastics is discussing with Celgene the purchase of commercial-scale units for the Mount Vernon unit. Although Celgene is initially targeting methylene chloride treatment for commercialization of the technology, the firm says the

process is applicable to other products, including aromatics, other chlorinated solvents and ketones. (Source: *Chemicalweek*, 20 November 1991)

Patching up the prairie with microorganisms

The vast prairies that once covered the Midwest are mostly gone now. But ecologists studying the vestiges of this ecosystem are finding new species that may prove useful.

In one patch of restored prairie, Wayne Nichols, professor of biology at Washington University, has discovered more than 500 species of algae, most of them new to science. One, *Sphaerellocystis aplanosporum*, performs the unique task of removing iron from the soil. This iron-eater also can extract the metal from laboratory cultures and select iron from among a group of elements. Another species craves silica. Its presence helps make clay soils more malleable. Nichols is cataloguing these new species in a comprehensive database that also characterizes the enzymes in the tiny organisms and how they react to antibiotics. Ultimately, say researchers, science could put these newly discovered species to work: the iron-eater might be used to help balance soils with too much iron, or to clean up contaminated soils. (Source: *Business Week*, 4 November 1991)

Calculating the value of harvesting medicinal plants in rain forests

In an effort to find new economic incentives for saving tropical forests, Robert O. Mendelsohn, associate professor of forest policy at the Yale School of Forestry and Environmental Studies, and Michael J. Balick, director of the Institute of Economic Botany at the New York Botanical Garden, have estimated the net income to be earned from harvesting medicinal plants in Belize.

Local herbal pharmacists and healers use medicines extracted from tree bark, leaves, vines and plant tubers to provide about 75 per cent of the rural population's health-care needs in the small Central American country.

The researchers estimate that net income from harvesting medicinal plants could exceed net income from farming or logging. These estimates do not take into account the growing interest among US pharmaceutical firms in using tropical plants to develop cancer drugs and other medications. New automated screening techniques have made it easier to analyse thousands of plant samples quickly. If useful chemicals are discovered, the demand for native forest products could rise.

By focusing on economic incentives, forest conservationists are "attempting to offer something in return for national efforts to preserve habitats containing thousands of rare plant and animal species. Harvesting marketable products from rain forests offers a realistic compromise between those who argue for forest preservation and those who want to give economic development first priority", says Mendelsohn.

In the cover articles of the March issue of *Conservation Biology*, the two researchers calculate that harvesting medicinal plants can yield an annual net revenue in the local markets of Belize of between $564 per 30 hectares and $3,054 per 50 hectares (one hectare equals 2.47 acres). Their calculations are based on a local wage of $12/day and assume that areas where trees and plants are harvested will be rotated, giving the harvested areas time to grow back. Allspice, copal resin, chicle and lumber could also be harvested.

The asset value of using the land to harvest medicinal plants is $3,327 per hectare, as compared to an expected value of $3,184 per hectare for proposed pine plantations from which lumber would be harvested on a rotating basis. In another study of cleared Brazilian rain forests, intensive farming ranked a distant third, with a land asset value of only $339 per hectare, according to Mendelsohn.

Professor Balick added that "a critical priority in rain forest conservation is to identify economically viable production systems that will enable small farmers to earn a good income and feed their families. Harvesting medicinal plants can provide a real income to farmers working small plots, which makes medicinal plants a resource worthy of protection and further study".

Although many people are sceptical about the effectiveness of the traditional medicines used in many Latin American countries, a large percentage of rural populations - hundreds of millions of people in the developing world - rely on these traditional medicines from the rain forest, according to Mendelsohn. One would therefore predict that their value would increase over time as tropical forests become more scarce.

Medicinal forest plants used by healers in Belize include China root, used for treating anaemia, rheumatism, acid stomach, skin conditions and fatigue; cocomocca, used for urinary tract and bladder infections as well as coughs and colds; and negrito, used to heal dysentery, diarrhoea and skin conditions, and as a tonic for the stomach and bowels. (Source: *ISEE Newsletter*, June 1992)

Ecologic bioprocessing - chances in new applications

A two-day meeting was organized by the Austrian Association of Bioprocess Technology (ÖGBPT) on behalf of the EFB Task Group on "Ecologic Bioprocessing". It was supported by the International Organization of Biotechnology and Bioengineering (IOBB), the Austrian Society of Biotechnology, the

Academy of Graz and Federal Ministries of Austria. The purpose was to make a step forward in the direction of sustainable development, thus to clarify the new technology paradigm, where biotechnologies could play a central part. Thereby, new application areas for bioprocessing should be identified, showing the strong and also weak points especially in comparison to chemical processing.

About 35 participants from many European countries (The Netherlands, Denmark, Germany, France, Croatia, Hungary, Czechoslovakia, Romania, Slovenia, Austria) attended the workshop.

Summary of the most essential facts and results

Chemical versus biotechnical processing

1. Comparison/differences

Chemo-processing quite often has a low public acceptance due to the increasing pollution problems where non-degradable, often toxic "chemicals" affect the environment (air, water, soil) and human beings (food, health). They mainly are based on non-renewable raw materials and use a lot of energy.

Biotechnologies also exhibit quite a low public acceptance as they are often considered synonymous with genetic engineering nowadays. Generally, high-tech is the result of the existing industrial approach in our western-style society, where both chemical and biotechnical processing show quite a similar profile and attitude. Differences between the two technologies can be identified in the fact that biotechnology has also another dimension: (bio-)degradable products are formed which are environmentally friendlier. However, in this area, the approach is mainly a conventional end-of-pipe technology with restricted capacity of problem solution in general. This agrees with the still dominating mechanistic, reductionistic paradigm.

2. Future perspective

The public, however, and frontiers in science have already adopted a "new" world view, i.e. the ecological, cybernetic or holistic paradigm. Thereby, the structure changes from a mainly linear framework (clock-work as metaphor) to a network with dynamic interaction (organism as metaphor). The function also changes, implementing the capacity of self-organization, i.e. evolutionary capability. The consequences of these changes in thinking and behaving are already manifest in a great number of people who apply common sense in following "new ethics" and in the field of restructuring organization (e.g. management in industry). Based on the changing values we can also anticipate a restructuring of technology in the next decade: technology-innovation will increasingly be based on the same principles, which can be spiritually extracted from ecosystems; therefore they are called "ecological principles". They can be used as a general guideline for restructuring and can be termed "sustainability". Sustainability has to be seen in the context of a long-term strategy, where current natural needs of mankind can be fulfilled without jeopardizing the prospects of future generations. Thus, the problem-solving capacity is higher in the new paradigm.

3. Ecologically sustainable technology

After handling the question of why a change in technology is needed and what is wanted by the public, now the question will be answered of how the restructuring/innovation of technology should be implemented. Sustainability will be the key. It includes a series of working principles, which can be derived: embeddedness of technosphere into biosphere, i.e. closed cycle production with renewable raw materials and non-toxic bio-compatible catalysts and product; failure-friendliness technologies; more cooperation than competition, more decentralization and locally adapted development resulting in a series of "technology mix" in regional niches on a small or medium scale based on old indigenous practices in full harmony with nature fulfilling the local needs first; holistic approach by including ecological research, e.g. in the case of genetic work and also by including the external costs for environment in the economic accounting; prevention is superior to intervention or end-of-pipe actions. Full attention is to be paid to the period of transition towards sustainability. Thereby external factors become important (public awareness push, new economic accounting, e.g. eco-national-product, available human capacity for sustainable development). The new concept of ecologically sustainable technologies can be realized in both areas of chemical and biotechnical processing (see article by A. Moser in *BFE*, Vol. 8, issue 11).

New areas of ecologically sustainable biotechnologies

Up to now a series of appropriate processes already exists and some of them exhibit good chances for realization and will replace non-sustainable (chemo) processes: e.g. biopolymers, biological control agents (biopesticides, -herbicides, -insecticides), biofertilizers, bioleaching and biosorption (biohydrometallurgy), biodesulphurization of coal, biodenitrification of ground and drinking water, biodepolymerization using lignocelluloses and wastes, biodegreasing, biodchairing, bioremediation including bio-soil-decontamination and enzyme technology in general etc. and intelligent niches like biodrugs from plants, bioflavours and biocosmetics and bio-odours. The competition with chemo-processing in general, however, can only be successful if efficiency of existing operations of bioprocessing is increased by using e.g. higher biomass concentration, integrated processing, less water, etc. The sustainability of renewable raw material-based production of

bulk-chemicals or bulk-"biologicals" is also still to be proved, depending on the introduction of ecologically sound economy measures.

Some of the cases listed above will be chosen in the next period of the EFB Task Group as test cases in order to demonstrate more quantitatively and "objectively" the potential of the new ecologically sustainable technology paradigm illustrating some essential facets of the biospheric compatibility and the validity of shown "ecological principles". Thereby, the uniqueness of biotechnologies in the direction of a sustainable world will become clear together with guidelines for research and development. (Source: *BFE*, Vol. 9, No. 1-2, January/February 1992)

Industrial equipment

Biosensors

Protein molecules encapsulated in transparent glass matrices could be the key to a new generation of biosensors, according to researchers from the University of California. Using techniques based on sol-gel technology, the team has developed a method for incorporating proteins into matrices without changing their reactivities or spectroscopic properties.

The sol-gel process is already used to manufacture silica glass by acid hydrolysis of alkoxide compounds such as tetramethylorthosilicate. After reaction with methanol, the resulting gel is carefully dried to remove the solvents, leaving an optically-transparent, porous glass.

Little or no heating is involved, so the technique can incorporate thermally sensitive molecules into glass matrices, but the acid conditions and alcoholic solvents involved would damage protein molecules. The new method involves adding a buffer solution, after hydrolysis and before addition of the protein, which raises the pH to a level which will not denature the protein.

The group tested the new method on metal-containing proteins such as copper-zinc superoxide dismutase (CuZnSOD) and myoglobin. These molecules have colours and electronic absorptions that can be studied easily. The electronic spectrums of the encapsulated proteins were unchanged. The team carried out reactions such as removal and replacement of the metal ions from CuZnSOD, by soaking the glasses in the appropriate reagents. The results were identical to reactions carried out in solution. (Source: *Chemistry & Industry*, 16 March 1992)

Biosensors in health-care applications

By the end of the decade, the confluence of biotechnology and microelectronics will produce a host of cheap, disposable biosensors. Such is the word from Japan, where biosensor research is marching steadfastly into the future.

Company	Biosensor R&D Activity
Dainippon Printing	Immune-system monitoring
INAX	*In vitro* measurement of albumen in urine
Itoh	High-sensitivity meat freshness
Nichirei	Optical measurement of fish freshness
Nippon Denso	Measurement of lactic acid and ammonia in perspiration to determine fatigue
Nissin Seifun	Fruit ripeness
Seiko Denshi	Artificial pancreas combining biosensor and micromachining
Takenak Kohmuten	Environmental pollution monitoring
Toto	Health and medical monitoring

Source: *Weekly Diamond*.

"In Japan", notes Isao Karube of Tokyo University's Research Center for Advanced Science and Technology (RCAST), "the main application field will be health care, for which companies like INAX and Toto are developing disposable biosensors". Initially, says Karube, commercial biosensors will be used in medical diagnosis and treatment. Subsequent biosensor applications will be found in food production and environmental monitoring. "Furthermore, telemetric biosensors for monitoring fatigue could be of interest in sports and athletics", Karube adds. "In the home, sensors for odour, freshness, and taste of foods could be used to determine food quality."

Karube, one of Japan's leading figures in biosensor research, is renowned for the development of the Ion-Sensitive Field Effective Transistor (ISFET), a micro-pH device made with the technology used to fabricate silicon devices. Karube states that ISFETs are useful for the potentiometric function of microbiosensors

because of their sensitivity to ion concentration and charge. (Source: *Bio Technology*, Vol. 10, February 1992)

Extraction industry applications

Mining with microbes

Research laboratories around the world are witnessing a marriage between biotechnology and metallurgy, creating a new discipline known as biohydrometallurgy. This is not just a case of researchers trying to hitch a ride on the environmental bandwagon. The method was first used commercially in the late 1970s to extract the uranium left in old mines in Canada. At roughly the same time in South Africa it was applied in two gold mines.

Smelting copper by traditional methods had cost between $60 and $90 per pound. The introduction of biohydrometallurgy cut the cost to less than $30 per pound. Smelting one ton of copper typically results in two tons of sulphur dioxide being pumped into the atmosphere. Biological extraction avoids this.

Biohydrometallurgy is straightforward when applied to copper production. First, the low-grade ore - and tailings left from any earlier conventional mining - is piled up in an area where the ground has been made impermeable. It is then sprayed with a leaching solution that contains iron, in the form of the Fe^{3+} ion, sulphate ions (SO_4^{2-}) and *T. ferrooxidans*. These bacteria are "acidophilic autotrophs", meaning that they are able to live solely on sulphides and thrive in an acidic environment. The sulphate ions in the leaching solution form sulphuric acid, giving a suitably acidic solution.

Copper is released when *T. ferrooxidans* catalyses chemical reactions that yield copper (as Cu^{2+}), iron (as Fe^{2+}) and sulphate. Because the piles sit on an impermeable base layer, it is easy to drain off the solution carrying the copper; the metal is then removed from it with another solvent. The remaining leaching solution flows into an open pond, where *T. ferrooxidans* catalyses a reaction that oxidises Fe^{2+} ions to Fe^{3+} ions and reduces the sulphide to sulphate. This recharges the leaching solution, which is pumped back to the top of the pile for the cycle to begin again.

The copper, meanwhile, is extracted as sheets through an "electrowinning" process, in which electricity is passed through the copper solution. The metal collects on the negative terminals. This part of the process is still costly in energy, but research is under way to develop "bioabsorption filters" such as algae, which could be used to make the process entirely biological.

Biohydrometallurgy may provide a method of underground mining, without the environmental damage associated with conventional techniques. There is now a mine in San Manuel in Arizona, consisting of five holes drilled into an ore deposit, which was fractured by detonating an explosive charge underground. Instead of standard mining practice, a mixture of acidic water and *T. ferrooxidans* is pumped down the central hole where the bacteria do their work. The resulting solution, rich in valuable copper, is pumped from the other four holes, processed and recycled.

Despite the potential of these methods, the mining industry is reluctant to use them. So far, they have been applied only as a last resort to recover low-grade metals from sites where traditional methods are not profitable. The problem lies in the slowness of the biological processes.

John Madgwick and his team at the School of Biotechnology at the University of New South Wales is trying to speed things up. He has been working on the extraction of manganese by so-called heterotrophic leaching, a process carried out under air-starved conditions similar to the anaerobic fermentation stage of brewing. By adding carbo-hydrate in the form of sugar to a solution containing an ore, Madgwick speeded up significantly the extraction of manganese. Heterotrophic organisms, unlike autotrophs, feed on carbohydrates produced by other plants and animals. In this process, according to Madgwick, "the mineral itself becomes a substitute for oxygen, acting as an electron acceptor at the end of an oxidation of sugar". It is possible that genetically engineered microorganisms could make such reactions run even faster.

A second feature of Madgwick's research brings a bonus for biohydrometallurgy in general. As well as finding more efficient methods of extracting manganese from its ore, he and his team are also finding ways to remove manganese from the separated solution. This process, bioabsorption, uses bacteria and algae to oxidize dissolved manganese. As well as separating metals for commercial purposes, this method might also be used to filter low concentrations of polluting heavy metals from waterways.

Microorganisms are also involved in naturally occurring electrostatic processes that alter the concentration of minerals, with potentially profitable results for humanity. Many microorganisms have a cell envelope or sheath that can absorb a wide range of metals and other toxic materials, even when they are present at very low concentrations. These bacteria, which are common constituents of soil, may play an important role in the formation of some gold and silver deposits in sediments laid down by rivers - alluvial deposits. In 1979, W.C. Ghiorse and P. Hirsch of the Idaho National Engineering Laboratory in Boise demonstrated that metals continue to accumulate on the

negatively charged polymers even after the bacteria have died; the process could continue indefinitely. There is even speculation that large gold nuggets could have formed in this way.

If biological processes are important in producing the millions of tons of alluvial gold, silver and other metals that generations of miners have panned from rivers and streams, it would make sense if mining engineers had some biological education.

Political pressure on the metal industries could force the pace of change. Widespread application of these new techniques may come from research carried out at the Idaho Laboratory, using microorganisms like *T. ferrooxidans* to remove the sulphur from coal. Extracting sulphur in this way could be a relatively cheap way of reducing the emissions of sulphur dioxide from coal-fired power stations. Applied in countries such as India and China, where industry is developing rapidly, this technique could bring enormous environmental benefits.

Microorganisms are already proving to be cost-effective as biological filters to remove heavy metals and other toxic material from polluted water. Rushes such as cats'-tails (*Typha latifolia*) collect microorganisms that metabolize heavy metals in the sediment around their roots. By selecting plant species which would absorb low-level pollutants, the town of Arcata cleaned up its water enough to render fish from Humboldt Bay edible for the first time in decades. As a bonus, the wetland has turned into a major bird sanctuary and tourist attraction. And if the polluting heavy metals can be extracted and used again, dealing with waste water in this way could make a profit.

There are signs that such extraction could succeed. Madgwick, in conjunction with the giant mining company BHP, is considering the use of single-celled algae to target specific metals in waste water. Harvesting algae rich in zinc, mercury or manganese, for example, could make the process of cleaning up water cost-effective or even profitable. And if miners can extract metals from ores of lower grade than is possible with conventional techniques, they can transform old tailing sites from polluting nuisances into valuable sources of raw materials. But research on biological techniques of metal extraction remains rare. Industry has funded much of the research in biohydrometallurgy, which is now beginning to pay back the investment. If society wants to make mining protect the environment, industry's demand for profit will mean that we may have to accept higher prices. But increasing environmental concern, coupled with the cost-effective techniques of biohydrometallurgy, could change the face of the mining industry for good. (Source: *New Scientist*, 4 January 1992)

Bacterial gold

Fine grains of gold "panned" from streams are at least partly a chemical precipitate on bacteria, claims a geologist in the United States.

John Watterson of the US Geological Survey in Denver caried out a study of tiny gold grains, known as placer gold, from Lillian Creek in Alaska. When he examined them under a scanning electron microscope, he found lace-like networks which he claims resemble the budding structures of the common bacteria *Pedomicrobium*.

The gold grains were less than 0.1 millimetre across. Watterson says that particles which are larger are much more likely to have had any fine lacy structures damaged.

It is not clear how gold might collect on the surfaces of bacteria. But Watterson thinks it may be a chemical residue which is left after bacteria break down humic acids in the Alaskan soil. Such acids contain gold. Another possibility is that the gold comes from the enzyme activity outside the bacterial cell. One species of *Pedomicrobium* is known to form manganese oxide on its surface in a similar way.

Watterson believes that most placer gold from Alaska may have collected on bacterial surfaces. But the process that produces the particles is not limited to Alaska or to cold environments. Similar lacy gold particles have been found in Chinese rocks, which are 220 million years old, and in South African rocks that were formed 2.8 billion years ago. However, the gold could have been a later addition, says Watterson.

He says that one day it may be possible to use bacteria to collect gold commercially. However, no one is going to get rich quick. A gold grain 0.1 millimetre across takes at least a year or so to grow. (Source: *New Scientist*, 2 May 1992)

Uganda may adopt cobalt bioleaching

If plans put forward by BRGM, the French State-owned mining group, and Barclays Metals, the metals trading subsidiary of Barclays Bank, go ahead Uganda could end up producing around 5 per cent of the Western world's output of cobalt. The project would represent the first time that bioleaching based on *Thiobacillus ferrooxidans* bacteria has been used to win cobalt.

The bacteria, already used to extract copper, gold and uranium, would be used to produce 1,000 tons a year of cobalt at an estimated cobalt price of $10-$12 a pound. The partners believe that bioleaching

could be the most environmentally acceptable method of extracting the cobalt from the 1.1 million tons of pyrites stockpiled at the old Kilembe copper mine on the edge of the Queen Elizabeth National Park in south-west Uganda. If all goes well, a $4 million feasibility study would lead to the construction of a $50 million bioleaching plant at Kilembe. (Source: *Biotechnology Bulletin*, February 1992)

Bio-hazards

Clean white coats spread mutant microbes

A group of Dutch researchers says that laboratory coats intended to protect biotechnology researchers provide the perfect escape route for large numbers of possibly dangerous, genetically engineered bacteria.

Most experiments and commercial processes in genetic engineering are based on the bacterium *Escherichia coli*, particularly a strain called K12. Opponents of biotechnology argue that *E. coli* primed with unnatural genes could be dangerous if they escape and pass the genes to other bacteria.

Biotechnologists have always replied that K12 can survive only in laboratory cultures and any that cling to a wet lab coat soon die when the coat dries. The flaw in the system, say Hayo Canter Cremers and Herman Groot, of the Dutch National Institute of Public Health and Environmental Protection, is that laboratories send the white coats to ordinary local laundries.

The Dutch scientists found that the bacteria survived on wet coats that dried at the laboratory before being sent off to be washed. They isolated perfectly viable *E. coli* from dried coat material. This undermines all the attempts to keep the bacteria in the laboratory, because the first step of the commercial wash is to soak the coats in water at 35° C - just the right condition to release the bacteria. The water, with added *E. coli*, is flushed straight into the sewerage system.

In tests on snippets of a two-year-old laboratory coat, K12 survived just as well as the hardier wild strains of *E. coli*, even when the K12 was carrying an introduced stretch of foreign DNA. Cremers and Groot say that white coats are regularly infected with genetically modified *E. coli* during normal work.

The team also found that the bacteria penetrate between the fibres of the cloth and probably infect clothing beneath. Most people wash their clothes at a temperature between 40° and 60° C - and the researchers isolated live K12 from wash water at this temperature. This strongly suggests that *E. coli* on clothing will enter the sewerage system, they say. K12 can survive for 72 hours in sewage. (Source: *New Scientist*, 21 March 1992)

Biotechnology policy and the CGIAR: seminar on biosafety and intellectual property rights

To discuss the key principles of future policy on new management aspects that modern biotech-nology brings along - biosafety and intellectual property rights was the primary purpose of the seminar *Biotechnology policy and the CGIAR*. The meeting took place 2-4 September 1991, organized by the Taskforce on Biotechnology (BIOTASK) of the Consultative Group on International Agricultural Research (CGIAR). It was attended by some 70 participants from international agencies, International Agricultural Research Centres (IARCs), universities, public institutes, and private companies.

The seminar was held at the International Service for National Agricultural Research (ISNAR, The Hague) and was financed by the Dutch Directorate General for International Cooperation. The CGIAR considers system-wide and transparent policies on biosafety and intellectual property rights as very important, to facilitate the acquisition of new biotechnologies by its centres, and to raise public awareness of these issues. The seminar was also intended to identify more clearly the needs of National Agricultural Research Systems (NARS) in these areas, as their position might differ from those of the international institutes.

International property rights

It will probably take much longer before agreement is reached upon a CGIAR policy with respect to Intellectual Property Rights (IPR). The discussion on IPR management was built around a working paper of Prof. J. Barton (Stanford University, USA) and Dr. W. Siebeck (World Bank), providing an extensive overview of all the issues involved in developing IPR policies and procedures.

Presentations on national and regional approaches to IPR revealed that many developing countries are likely to adopt or strengthen intellectual property protection over the next years. IARCs are also often recommended to actively seek patents for their inven-tions, in order to facilitate collaboration with the private sector, to prevent the appropriation of inventions by others (defensive patenting), and eventually to seek earnings from royalties. However, two major concerns were expressed. Firstly, IPR in developing countries will probably have a limited, or even negative, effect on local innovation efforts. Moreover, the effect of IPR on the transfer of new technology to developing countries may also be limited, or negative. Secondly, a policy on IPR

will substantially modify the CGIAR's current open-door policy, meaning the free distribution of plant genetic material and innovations.

The formulation of a general CGIAR policy will also be hampered by the diverging interests of different IARCs. For the commodity centres, a critical factor in determining their policy stance on IPR derives from the existence, or absence, of private breeding activity for their mandated crops. This situation is different for crops such as maize, in which the private sector is conducting large-scale research, as contrasted with crops such as cassava, for which there is essentially no private research. In the case of cassava, for instance, there is no need for defensive patenting, and restrictions associated with acquiring new technologies will be minimal. Individual IARCs will therefore probably formulate their own policy on IPR. In the critical publication *Patenting life at the IARCs*, the Genetic Resources Action International Network (GRAIN) reports that ICRISAT has already drafted a proposal for the establishment of IPR administration at that centre. The draft follows the recommendations of a workshop on this theme which was held in November 1990 at ICRISAT. The draft proposal is very much directed at facilitating collaborative research with the private sector in industrialized countries, without taking into account the probably differing interests of other partners of the IARCs, such as the research institutes in developing countries. Also, the proposal implies a radical break with the open-door policy. ICRISAT's administration of IPR would mean that the dissemination of technology and germplasm will be controlled by licensing agreements, instead of distributing it for free.

Following the seminar, a CGIAR committee prepared a first policy draft on IPR. After consultation during the International Centres Week (ICW), it was decided to prepare a paper on an integrated CGIAR policy on biodiversity, IPR and biosafety. (Source: *Biotechnology and Development Monitor*, No. 9, December 1991)

E. PATENTS AND INTELLECTUAL PROPERTY ISSUES

PCR regulations

Following up on its promise to relax licensing restrictions on the use of the polymerase chain reaction (PCR), Hoffmann-La Roche has released new regulations affecting academic and non-profit laboratories using PCR to diagnose human disease.

The new agreement grants these laboratories the rights to perform tests for a wide variety of diseases and conditions, such as infectious and genetic diseases, genetic predisposition to diseases, tissue-transplant typing, and parentage determination. Under the old agreement some of these applications - including the use of PCR for HIV detection - were forbidden. The company will maintain its policy of charging no up-front licensing fees, and royalties will drop to as low as 9 per cent. Royalty rates under the old agreement are "confidential, but they were higher than 9 per cent", says company spokeswoman Paula Evangelista.

Licenses to use PCR for research, rather than commercial diagnostic purposes, cannot be obtained through Roche. The exclusive distributor for non-diagnostic applications is Perkin-Elmer Corp. in Norwalk, Connecticut. (Source: *Science*, 21 February 1992, p. 927)

NIH defends gene patents as filing deadline approaches

The US National Institutes of Health (NIH) vigorously defended its approach in spite of the concerns of its government partner in the US genome project and criticism from other countries.

Speaking at a public meeting of an interagency committee that is formulating a US policy on gene patents, Bernadine Healy, the NIH director, repeated that NIH was following both legal precedent and a legislative mandate to commercialize its inventions when it filed a patent for more than 2,000 partial cDNA sequences last summer. She noted that the two main associations representing virtually all the US biotechnology industry - the Industrial Biotechnology Association (IBA) and the Association of Biotechnology Companies (ABC) - support the NIH patent application.

The meeting was ostensibly to solicit public input for the deliberations of the committee, a genome patent working group that serves under the Federal Coordinating Council for Science, Engineering and Technology within the White House.

The working group intends to make sure that the Government does not repeat its mistake of making a decision on this subject without getting input from every possible source. European and Japanese patent law gives the United States a 12-month grace period after a US patent application in which to file elsewhere. For NIH that deadline arrives on 20 June, and US officials promise that they will have adopted a coherent policy by then. Deciding not to file in Europe and Japan would be a policy reversal nearly as significant as the initial decision to file.

In a letter sent to President George Bush, the ABC supported the NIH patent application and future sequences as "essentially the only responsible course under existing federal law" while the issue of patentability remains open. Following the IBA, which drafted a similar statement earlier this year, the ABC called for NIH to work with industry on a licensing policy for the sequences.

Given that a great deal of work is needed to turn a partial cDNA sequence with unknown function into a marketable product, the ABC asked NIH to license the sequences non-exclusively. (Source: *Nature*, Vol. 357, 28 May 1992)

Japanese researchers rule out gene patents

The leading scientists in Japan's effort to sequence the genomes of humans and plants say they do not plan to join the United States and Britain in filing patents on their discoveries but will instead publish the data and make it freely available. The various government ministries and agencies involved in genome research have yet to develop a policy on the patenting of genes, and it would be unprecedented for the Government to intervene in a scientific decision of this type.

A controversy erupted among genome researchers last year after Craig Venter of the US National Institutes of Health (NIH) filed patent applications for more than 300 cDNA fragments of human genes. Researchers at Britain's Medical Research Council (MRC) initially condemned Venter's move, but the MRC has since decided to file patent applications for more than 1,000 cDNA sequences.

At present, only the Science and Technology Agency, which is expected to play a leading role in Japan's human genome project, has established a small committee of five lawyers to examine the issue of patenting human genes. But the committee is not expected to produce a report for at least a year.

The Japanese group uses sequencing techniques that are similar to what is done by the NIH team, but the goals of the two teams differ somewhat. (Extracted from *Nature*, Vol. 356, 19 March 1992)

Progress on animal patents

A draft European Communities (EC) directive that would endorse the patenting of transgenic animals has finally cleared its most important hurdle. After nearly three years of inactivity, the European Parliament's legal affairs committee has decided that the "inventors" of transgenic animals should be able to file patents in the EC. European Commission biotechnology officials believe that the directive, first proposed in 1988, will now be adopted by ministers from the EC member states before the end of 1993.

The directive has been controversial since its inception, attacked by the European Green movement and animal rights groups, who argue that many transgenic animals, particularly those produced for commercial gain, are likely to suffer. (Extracted from *Nature*, Vol. 355, 30 January 1992)

US to press for global patenting?

In April two bills were introduced to the US Senate and House of Representatives with the objective of harmonizing global patents.

Among the major thrusts of their presentation is the need to protect US inventors from losing patent rights outside the USA by neglecting existing differences. Most obvious is the current US principle of First to Invent. By supporting his claim to invention with notarized laboratory notebooks or other equally compelling evidence, the inventor can patent his invention with precedence over other filers who may have filed first but cannot prove precedence.

In Europe First to File gets the patent - inventors beware! If you publish your invention as part of a scientific paper the invention becomes public knowledge and unpatentable. Fast competitors can modify the invention and make their own claims which strip the original inventor of all rights. In the USA the year of grace allows freedom to publish without compromising patentability. In the UK, the Science and Engineering Research Council, SERC, has been pressing recently for acceptance of the year of grace principle. The new US bill will be warmly welcomed. (Source: *BFE*, Vol. 9, No. 6, June 1992)

New international patent application procedures

The Assembly of the International Patent Cooperation Union has adopted changes to the regulations of the Patent Cooperation Treaty (PCT). The changes are designed to streamline the patent filing procedures used to obtain international patents.

The PCT offers inventors and industry a simplified procedure for multiple filing of patent applications in 49 countries. An international application can be filed in the applicant's own language, with the home patent office acting as the receiving office. Details from: World International Property Organization, 1211 Geneva 20, Switzerland. (Source: *Biotechnology Bulletin*, January 1992)

Australia says "yes" to biotechnology patents

An Australian parliamentary committee says that the Government should permit the patenting of live organisms and that any objections to genetically modified organisms should be dealt with by other means. The report, by the Standing Committee on Industry, Science and Technology, is not expected to be challenged by the rest of Parliament. Its significance is heightened by the fact that Australia is the only country so far to approve such a live organism for general release.

The committee's report concentrates on setting guidelines for the conditions under which such organisms should be used. It recommends that existing, semivoluntary approved processes be strengthened and given the force of law. It also calls for a formal mechanism to obtain approval for the release of both live organisms and those that are by-products of such genetic engineering. (Source: *Nature*, Vol. 356, 2 April 1992)

Patents thwart genome project

So long as the UK continues to patent sequences of DNA, scientists in Italy, Germany and France are refusing to place DNA sequences in a database in the UK.

The Medical Research Council has filed applications to patent more than 2,000 sequences of human DNA known as expanded sequence tags. These DNA fragments have a recognizable sequence of bases at one end but no known biological function. They are used in mapping sequences of longer streiches of DNA. Six months ago, the European Commission started funding a database so that European scientists could store data on these sequences and compare notes.

The database is located at the MRC's unit at Northwick Park in London. But so far, German, French and Italian participants in the scheme have refused to add their sequences to the database. Participants in the project blame the MRC's application to patent its sequences at the US Patent Office. Until the patents are approved or rejected and the sequences are published, other participants will not know whether their fragments are the same as those the MRC wants to patent.

The MRC itself disagrees with patenting genes for which no function is known, but because scientists at the National Institutes of Health in the US are doing so, the MRC feels obliged to patent in order to protect its interests. (Source: *New Scientist*, 11 April 1992)

Gene patent move by NIH viewed as overreaching

National Institutes of Health filed patent applications for 2,375 human brain genes. NIH patented 347 genetic fragments a year ago and is now laying claim to roughly 5 per cent of all human genes, but the Government's efforts are drawing fierce criticism from scientists and have fuelled an international debate over patent rights and the future of biotechnology.

Researchers accuse the Government of acting prematurely. They say NIH does not yet understand the roles the genes play in the body and patenting them could block research and development of new drugs and therapies.

Patent lawyers question whether NIH's efforts can stand up in court. The Government does not know the biological roles of the genes it has patented, but J. Craig Venter, the NIH scientist in charge of patenting the genes, says knowledge of the fragments is specific enough to warrant the patents.

Scientists also worry that the Government may set off a patent race among nations and firms. NIH takes copies of deoxyribonucleic acid fragments and determines their molecular sequence. The DNA fragments are then tested to make sure human cells can decipher them, thereby proving that they are either genes or parts of genes. (Source: *Chemical Marketing Reporter*, 17 February 1992)

Challenge to forensic database

A UK civil rights group, Liberty, is seeking to take London's Metropolitan Police to the European Court of Human Rights, questioning the legality of its database of DNA fingerprinting results. The civil rights group Liberty's argument turns on the case of Roy Williams, a man questioned during a 1988 murder inquiry and subsequently cleared after voluntarily submitting a DNA sample for analysis - but who later found that his DNA profile had been included in the Metropolitan Police database without his consent. If taken up by the European Commission for Human Rights, the case may take as long as five years to resolve, but Liberty's action highlights a debate likely to gain momentum over the coming year, as national DNA profile databases come into general use in Britain and the United States. In Britain, the Home Office Forensic Science Service has set up a central database which police forces can ask to be trawled for matches to their scene-of-crime samples, and the Federal Bureau of Investigation (FBI) expects to have a similar US database in place a year from now. (Extracted from *Nature*, Vol. 355, 16 January 1992)

Courtroom battle over genetic fingerprinting

Sloppy laboratory work creates the greatest risk of error when samples of DNA are used as evidence in court, according to a new report from the National Academy of Sciences. The academy calls DNA analysis a "powerful tool", but it also calls for caution in interpreting DNA results and regulation of laboratories that carry out analysis of DNA.

Supporters and opponents of DNA "fingerprinting" in the US have been fighting an increasingly bitter battle in courtrooms, scientific journals and the media. The report recommends regulation and testing of all examiners and laboratories that produce evidence for use in court. Outside inspectors should conduct this "blind proficiency testing", and the results should be made public.

The FBI should change the way it calculates the probability that a random person's DNA would match samples found at the scene of a crime, says the report. The new procedures would increase the estimated

probability of a chance match from 1 in many millions to 1 in perhaps 10,000.

The committee called for detailed study of 15 to 20 different ethnic groups in the US. Two people from the same ethnic group are more likely to have a close match, yet in court cases, the likelihood of a random match between DNA from two different people is usually estimated using data from the general population.

A new analysis of genetic data from an isolated indigenous group in South America produced a dramatic illustration of this point. (Extracted from *New Scientist*, 18 April 1992)

Plant varieties patentable in Mexico

The new Mexican *Law for the Promotion and Protection of Industrial Property*, effective as from 28 June 1991, follows the new minimum patent standard aimed at by industrialized countries in international negotiations in the World Intellectual Property Organization (WIPO) and the General Agreement on Tariffs and Trade (GATT). With this new law, Mexico is one of the first developing countries that **explicitly** provides patent protection in the realm of biotechnology. Focusing on how Mexico has come this far results in a story on the efficacy of the American trade pressures. (Extracted from *Biotechnology and Development Monitor*, No. 9, December 1991)

New international database

By 1993 the public will have access to a computerized database of all internationally issued patents and published patent applications covering specific nucleic acid and amino acid sequences.

This system will replace the current, laborious method of searching for prior art in paper form and comparing sequences by eye. The program, which is expected to be made available on compact disc (CD) and through on-line services, will automatically compare sequences for the user.

The decision was made final in late February, when representatives from the USA, Japanese and European patent offices signed the Ninth Memorandum of Understanding on Trilateral Cooperation at the Ninth Annual Trilateral Conference in Tokyo.

Under terms of the agreement, each patent office will provide the others with computerized, digital images of back-files of all original patents issued, in the case of the USA, and patent applications originating in Japan and Europe, to be made available publicly in these countries by the end of 1993.

The agreement states that any third party may purchase the database in any format from any of the three patent offices for the marginal cost of preparing it. No development costs will be built into the fee. (Source: *Genetic Engineering News*, 15 March 1992)

Micro-organism cultures isolated from sewage are patentable

In a decision interpreting Section 7(2) of Israel's Patents Law, which excludes from patentability any new animal variety "except microbiological organisms not derived from nature", the Commissioner of Patents held that biologically pure cultures of micro-organisms isolated from sewage are patentable, as they are not "derived from nature". (Patent Applications Nos. 75013 and 86190)

In a previously decided case, pure cultures of micro-organisms discovered in waste water purification plants in Switzerland and Tunisia were held to be patentable, on the ground that waste water purification plants were not to be considered as "nature". In that decision, the Deputy Commissioner stated, *obiter dicta*, that mere purification does not render a naturally occurring micro-organism patentable.

In the present case, applicants' major contention was that pure cultures of micro-organisms derived from nature should be patentable, since the very act of selection and purification of a single clone of micro-organisms results in a product which did not exist in nature in that form. Applicants pointed to the high rate of spontaneous mutations in micro-organisms and the large number of generations through which the micro-organisms propagated during purification and selection.

In the alternative, applicants argued that the case was governed by the company and that the claims should be allowed on the ground that sewage may not be considered "nature".

The Commissioner dismissed the main plea, holding that in the absence of concrete proof that the isolated micro-organisms had not existed in nature, a pure culture of a micro-organism derived by known selection and purification methods from a sample found in nature was still a micro-organism derived from nature and therefore was not patentable under Section 7(2). However, the Commissioner accepted the alternative argument and allowed the claims on that basis. (Source: *World Intellectual Property Report*, 1992)

F. BIO-INFORMATICS

Biotechnological Innovations in Animal Productivity

Despite the difficulty of predicting the precise impact biotechnology will have on agriculture, many believe it represents a means for pivotal change leading to an extension of the scope and efficiency of agricultural production. This book and its companion BIOTOL volume *Biotechnological Innovations in Crop Improvement* explain the application of biotechnology to improving agricultural productivity.

The application of biotechnology to animal production is examined. Animal production, of course, depends upon the reproductive capabilities of animals, their growth rates and the ability of the farmer and veterinary services to prevent and cure infection. This book focuses onto these facets of animal production by explaining how the growth and reproduction of livestock may be manipulated and how diagnostic systems and vaccines may be developed using contemporary biotechnology procedures. The whole tenor of the text is on the application of biotechnology and it has been assumed that the reader is familiar with the key stages in recombinant DNA technology and has a background knowledge of animal physiology, reproduction and metabolism. The sections on vaccines and diagnostics are written on the assumption that the reader has knowledge of the basic features of the immune response including the roles of B, T and antigen presenting cells, the major histocompatibility complex and is familiar with some of the terms that are applied (e.g. epitope, immunoglobulin, antigen) in this area of study. The text has, however, been provided with many helpful molecular and cellular "reminders" and these, together with its open learning and interactive style, will enable most readers to overcome any lack of experience in the key areas.

Progress in the application of biotechnology in the animal sector of agriculture is however being impeded by a lack of sufficient knowledge about the genetics and physiology of animals and by the pressure that arises from a lack of acceptance of this technology in some sections of society. The acceptance of this technology and its application to animal production will have a significant impact on the pace of development in this area. Key to the future development of biotechnologically aided animal production will be questions of safety and ethics. The book does not attempt to dictate the ethics of manipulating the growth and the reproduction of animals. It is up to the reader to formulate his/her own opinion on such matters. However, information on matters of safety are included. The main purpose of the text is to ensure that the reader recognizes the benefits that may arise from the application of biotechnology to animal production and understands the routes by which these benefits may be realized. The fears of many regarding the abuse of animals through the application of science and technology are often the result of lack of information or media "hype". By providing a description of the present state and the limitations of biotechnological applications to animal production, this text may also provide the reader with the knowledge to respond more fully to the anxieties felt by the "man in the street" about modern practices in animal production.

Published by Butterworth-Heinemann for the BIOTOL (biotechnology by Open Learning) project. For further information, see page 16.

Bio-pesticides in Developing Countries

This study by E. B. J. Van Latum and R. Gerrits of the Foundation for Ecological Development Alternatives (The Netherlands), provides an overview of the most important literature with regard to bio-pesticides in developing countries. The word *bio-pesticides* as used in this study refers to "plant-derived pesticides." The main aim of the study is to raise international awareness of the potentials of bio-pesticides; the need to install a database to stimulate and promote further research; and the importance of saving the genetic diversity of plants, particularly in the tropics where many plants with pesticidal properties happen to be found. The study also indicates criteria under which safer use of bio-pesticides can be reached internationally. Further, the study highlights problems in the registration process in Europe and North America of bio-pesticides originating from developing countries. The Netherlands are taken as an example, being the country with the most stringent pesticide registration.

This study, which is No. 1 of the Biopolicy International Series produced by the African Centre for Technology Studies, should also stimulate further research. Neem is taken as an example to illustrate the connection between research, production of the pesticide and its application. The study surveys the most important literature and principal handbooks on plant-derived pesticides. Based on this information, criteria for the safer use of bio-pesticides are developed to give guidance to the use of such pesticides in developing countries. Available from ACTS Press, African Centre for Technology Studies, P.O. Box 45917, Nairobi, Kenya. ISBN 9966-41-010-4. Price US$7.50.

Ecological Effects of Genetically Modified Organisms

(Proceedings of a national symposium organized by the Netherlands' Ecological Society in cooperation with the Provisional Committee on Genetic Modification, Amsterdam, held on 19 September 1991 and edited by Jaap Weverling and Piet Schenkelaars with a preface by G. P. Hekstra.). This book, however, follows an opposite tract, similar to the procedure in the Netherlands's Project on Ecological Sustainability of the use of Chemicals, PESC. Not the chemical (cf. the manipulated gene) is the point of departure, but the

processes that govern the stability of an ecosystem to resist intoxification (cf. resistance against gene invasions).

Genetic manipulation and conservation of biodiversity has long been on the international scientific and political agenda and featured prominently at the United Nations Conference on Environment and Development at Rio de Janeiro, June 1992. Diversity - from biologically active molecules to ecosystems - is a fundamental property of every living system. The exact meaning of all this diversity is not known. Is biodiversity merely a manifestation of the dynamics of complex, non-linear systems, far from equilibrium? Or is it that processes such as selection and competition, mould each species to function harmoniously with those surrounding them in an ecosystem?

Demographic and economic growth of the human species, especially over the last fifty years created large environmental changes, including massive species extinctions. Already now more than 40 per cent of the world's production is in one way or another used for human food, fibre and wood consumption. Extrapolations into the next fifty years only spell gloom, unless we drastically change destructive production and consumption patterns.

The "great extinction", as people from IUCN call it, is still to come. Mostly by habitat destruction (deforestation, desertification, overexploitation) and - to a lesser extent - due to pollution. Flora and fauna are increasingly under chemical stress as well. More recently, however, another risk appeared at the horizon: invasions of manipulated species in habitats where they can strongly influence indigenous species to the brink of collapse of viable populations. Can the risks be assessed and predictions made? What can we learn from other types of risk assessment?

In chemical and ecotoxicological risk assessment it is common practice to start the valuation with the intrinsic properties of the chemicals (quantitative structure-activity relationships, QSAR's) that qualify them to affect life molecules and their processing organisms, and continue with the analysis of foodweb relations in order to calculate risks for other organisms in the system, and ultimately judge on the risk for the system at large, taking extrapolation factors and uncertainty margins in the models into account.

It looks seemingly logical that in risk assessment of genetically modified organisms one should follow the same tract, starting from the properties of the manipulated organisms to the calculation of risk when released in the environment. The analysis should include pathways into the foodweb and take extrapolation factors and uncertainty margins into account.

The book covers: The ecological risks of genetically engineered organisms: An introduction into the problem (Piet Schenkelaars); Environmental risks policy on genetically modified organisms in the Netherlands and Europe (C. J. van Kuijen); Cause and effect in natural invasions (R. Hengeveld); Invasiveness of plant pathogenic micro-organisms (J.C. Zadoks and P.J.G.M. de Wit). Available from the Netherlands Ecological Society, Drenthesingel 11, 6835 HG Arnhem, The Netherlands.

The Gene Hunters: Biotechnology and the Scramble for Seeds

This book by Calestous Juma is interesting and occasionally provocative. The author is clearly wary of the extent to which multinational corporations have gradually acquired controlling interest in many sectors relevant to biotechnology and is skeptical of their claimed intentions.

The first two chapters give a brief history of the movements of genetic resources in the wider sense, and place plant collecting activities in the context of earlier, imperialistic expansions of agriculture into colonies.

The third chapter looks at international activities involving genetic resources, plant breeding and the Green Revolution. It questions the way in which industrialized nations have exploited their opportunities, while leaving unacknowledged, or giving only token recognition (but no long-term benefits) to the sources of much of the biological diversity that underlies the multinationals' financial success. Much is made of the various activities aimed at recognizing and protecting the rights of plant breeders - most of whom just happen to work for multinationals, in the North.

The fourth chapter addresses animal biotechnology and microbes via the Microbiological Resources Centres (MIRCENs), which are the gene banks of the microscopic world, but differ in that samples submitted for safekeeping, unlike plant genetic resources, are fully described before deposition in the collections. The author suggests that the MIRCEN network should be subsumed into the newly-fledged International Centres for Genetic Engineering and Biotechnology (ICGEBs). The chapter also considers biotechnology's long-term effects, emphasizing the potential for industrialized countries to develop substitutes for raw materials traditionally imported from developing nations.

Ownership of genetic resources is considered in chapter five, with discussion of patenting and plant breeders' rights and their applicability to life forms. Disparities in legislation are noted, together with recent developments in the United States. This is coupled with the GATT trade negotiations, implying a new form of protectionism.

Chapter six is devoted to a case study of Kenya's agriculture and germplasm's role in it, and illustrates how

historical developments have shaped the direction of present research and development in agriculture.

The book concludes by looking at the "way ahead" for Africa, and examining policy options.

Published by Zed Books, London, 1990, 288 pp., ISBN 0-86232-639-7 (hardback), 0-86232-640-0 (paperback).

Global Biodiversity Strategy: Guidelines for Action to Save, Study, and Use Earth's Biotic Wealth Sustainably and Equitably

As the extinction of plant and animal species proceeds at an alarming rate, an international team of experts has devised a new approach to safeguarding Earth's biological wealth while providing a way for species-rich countries to benefit from their genetic wealth.

An initiative involving scientists, government officials, representatives of NGOs, business people, and local and tribal leaders on nearly every continent, this report differs dramatically from today's typical *ad hoc* piecemeal reaction to environmental crises.

The *Strategy* outlines a systematic agenda of policies and actions that can be adopted at local, national and international levels. With 85 specific recommendations, it sets forth a conservation blueprint that protects biodiversity over the long term while mobilizing its benefits for food, medicines, chemicals and other necessities.

The book was produced by the World Resources Institute, the World Conservation Union and the United Nations Environment Programme, and is available from World Resources Institute Publications, P.O. Box 4852, Hampden Station, Baltimore, MD 21211, United States. $19.95 (pbk.).

Biodiversity: Social and Ecological Perspectives

This collection of essays from the World Rainforest Movement (WRM) is a critique of a study entitled *Conserving the World's Biological Diversity* published by the World Resources Institute, the World Bank, the International Union for Conservation of Nature and Natural Resources, and the Worldwide Fund for Nature.

The contributors say that while the crisis of biodiversity erosion is focused as an exclusively tropical and Third World phenomenon, the thinking and planning of biodiversity conservation is projected as a monopoly of institutes and agencies based in and controlled by the industrial North. "It is as if the mind is in the North, the matter is in the South; the solution is in the North, the problems in the South."

The authors acknowledge that the tropics are the cradle of the planet's biological diversity and that the multiplicity and variability of ecosystems and species that exist there is incomparable. However, they believe that the erosion of diversity is a crisis in the North as much as in the South.

And so, while the loss of biological resources continues through clearing and burning of forests, overharvesting of plants and animals and indiscriminate use of pesticides, the authors say the deliberate substitution of diversity by uniformity of crops, trees and livestock - through development projects financed by international agencies - has worsened the biodiversity crisis.

Heffa Schücking and Patrick Anderson analyse the dominant paradigm of conservation; Andrew Gray writes of his experience with indigenous peoples of Latin America; Larry Lohmann's article reflects the conservation strategies of the peasant and forest-dwelling communities in the case of Thailand; and Vandana Shiva attempts to show that production based on principles of uniformity is the biggest threat to biodiversity. Available from Zed Books Ltd., 57 Caledonian Road, London N1 9BU, United Kingdom, and 171 First Ave., Atlantic Highlands, NJ 07716, United States.

Biotechnology for Sustainable Development: Policy Options for Developing Countries

Biotechnology has become a subject for national and international debate, bringing into sharp focus the double-edged nature of technology. While planners and scientists recognize the potential application of biotechnology in meeting basic needs and enhancing global competitiveness, they are also concerned about the risks associated with the technology. The challenge is how to maximize the benefits of biotechnology while reducing its risks. *Biotechnology for Sustainable Development* by Norman Clark and Calestous Juma, authors of *Long-Economics*, examines this challenge and outlines an international research agenda for generating viable policy options for Third World countries.

Available from: African Centre for Technology Studies (ACTS) P.O. Box 45917, Nairobi, Kenya. Tel.: (254-2) 744047, 744095. Fax: 743995. US$12.00, Ksh. 100.00. ISBN 9966-41-009-0. 117pp.

Guías para la liberación en el medio ambiente de organismos modificados genéticamente (Guidelines for the release of genetically modified organisms in the natural environment)

In this book, the *Interamerican Study Group on New Biotechnologies* presents a framework for the evaluation of environmental risks of the release of genetically modified plants, micro-organisms and veterinary biologics. Their aim is to accelerate the development of national regulations in Latin America

and the Caribbean. The study group is formed by the Interamerican Institute of Cooperation in Agriculture. the Panamerican Health Organization, the Organization of American States, the International Office of Epizootics and the US Department of Agriculture. Their first meeting in 1988 in San José, Costa Rica, dealt with the regulation of the use and safety of genetic engineering techniques of rDNA technology.

The guidelines presented in this book are the results of the second meeting of the study group, in May 1990 in Brasilia. They are based especially on existing Canadian regulations, but adapted to the regional requirements. Unfortunately, the book pays no attention to which adaptations actually have been made.

The first part of the book consists of regulations for the evaluation and approval of both small and extended field tests and for the authorization of import and commercialization of new biotechnological products. There are a lot of references to the more detailed information in the appendices which together form the second and largest part of the book. These twelve appendices are translations of parts of original publications by *Agriculture Canada*, the *Organization for Economic Cooperation and Development* (OECD), and others.

Available from: Instituto Interamericano de Cooperacion para la Agricultura. P.O. Box 55-2200, Coronada, Costa Rica, May 1991, 145 p., ISSN 0534-5391.

Biotechnologies in Perspective: Socio-economic Implications for Developing Countries

Following the *International Seminar on Economic and Socio-cultural Implications of Biotechnologies* in October 1990 (Vezelay, France) this volume is a compilation of the 17 papers presented and edited by Albert Sasson and Vivien Costarini. The seminar addressed the social, economic and cultural implications of biotechnological innovations, adopting a future-oriented approach.

The extracts of the presented papers during the seminar covered the following research areas: biotechnology and economic restructuring, industrial biotechnology policies, rural labour absorption, the impact of biotechnology on international trade, biotechnology and the public sector, developing public-sector private enterprise links in biotechnology, biotechnology and changing comparative advantage, and the impact of biotechnology on African economies.

Most of the authors present case-studies on their country of origin. The studies address: India, China, Kenya, Thailand, Tunisia, South-East Asia, Australia, Venezuela and Zimbabwe. The studies show striking differences in the adoption and application of biotechnologies. The countries of the Pacific Rim and South-East Asia, including the *Newly Industrialized Countries* (NICs) give biotechnology R&D a high priority in their development strategies. Sub-Saharan Africa, on the other hand, is generally far behind.

According to Dr. I. Robertson (Zimbabwe) the application of biotechnology in Africa could be made possible with a: "busload of dedicated, committed, funded scientists, plus a container-load of equipment [that] could soon cope with the pressing problems, the crushing poverty and hunger, and the intellectual loneliness of the Third World."

Future oriented studies, UNESCO. Vendôme: Presses Universitaire de France. 166 p. ISBN 92-3-102738-7. Price: US$9.90.

Biotechnology of Cotton: Achievements and Perspectives

This paper by J. McD. Stewart provides a thorough state-of-the-art review of the various applications of biotechnology to cotton improvement, and discusses rather superficially some of the societal, environmental and regulatory issues involved. The author expects that the greatest impact of biotechnology in cotton production will be associated with the development of pest resistant varieties, through the insertion of the *Bacillus thuringiensis* toxin gene. Fibre quality parameters will be improved and modified to extend utilization beyond that for which cotton is currently grown. These will begin to appear for the producers around 1995 and the most significant will be widespread by the year 2000. ICAC review articles on cotton production research No. 3. CAB International [Wallingford, Oxon OX10 8DE, UK] and the International Cotton Advisory Committee [ICAC, 1901 Pennsylvania Avenue, N.W., suite 201, Washington D.C., 20006, USA]. 56 p. ISBN 0-85198-714-1. Price: UK£17.50.

Agricultural Biotechnology: Opportunities for International Development

Part of a Biotechnology in Agriculture Series available from CAB International, this book aims to review advances and current knowledge in key areas of biotechnology as applied to crop and animal production, forestry and food science. Some titles focus on individual crop species or group of species, others on specific goals such as plant protection or animal health, with yet others addressing particular methodologies such as tissue culture, transformation or immunoassay. In some cases, relevant molecular and cell biology and genetics are also covered. Issues of relevance to both industrialized and developing countries are addressed, and social, economic and legal implications are also considered. (CAB International, Wallingford, Oxon. OXIO 8DE, UK).

Innovation and Sovereignty: The Patent Debate in African Development

The book presents a new approach to the search for solutions to the current economic, ecological crisis in Africa. It stresses the pertinent role played by technological innovation and institutional reorganization in problem-solving. The authors, Calestous Juma and J. B. Ojwang, argue that the conventional explanation of international trade based on natural endowment and comparative advantage are too simplistic to provide policy guidance. This book marks a major turning point in public policy research and practice in Africa. US$18.99, UK£10.50, Ksh.150.00. ISBN 9966-41-000-7. 252 pp. African Centre for Technology Studies (ACTS). P.O. Box 45917 Nairobi, Kenya. Tel.: (254-2) 744047, 744095. Fax: 743995.

African Development Sourcebook

The African Development Sourcebook is based on a survey of 174 networks in sub-Saharan Africa representing a wide range of social, cultural and economic development sectors. It aims at providing information about the numerous development actors in Africa, and to encourage and facilitate communication and information sharing between development organizations and practitioners world wide. Through this publication, UNESCO offers a creative solution to the complex problem of development information exchange, and gives a clear insight into the national, regional and information contributions to development in sub-Saharan Africa. Available from UNESCO, Paris (France). 1991, 157 pp. (+ 8-page detachable questionnaire). 21 x 29.5 cm. ISBN 92-3-002736-7. 70 FF. Bilingual: English/French.

Agricultural Biotechnology: Issues and Choices. Information for Decision Makers

The book, edited by Bill R. Baumgardt and Marshall A. Martin provides background information on the socio-economic aspects of agricultural biotechnology. It is a reference source for agricultural professionals to better understand the issues at hand and helps tackle discussions with the public. Although it is mainly written from a US perspective, it still offers interesting information for Third World policy makers. Concern for the international competitiveness of US agriculture is present throughout this book.

The US system to monitor and regulate food and environmental safety could serve as an example for developing countries. An overview is given in chapter six. Intellectual property rights and the global perspective of agricultural biotechnology are dealt with in chapters seven and eight. According to the authors, the tendency in the USA to restrict the flow of biotechnological information, and the efforts to strengthen the protection of intellectual property rights in the developing countries, could work counterproductively. Hampering the diffusion of biotechnology to the Third World, could lead in the long-run to a slow-down of global agricultural and economic development.

The book proceeds with two chapters on ethics and values, and on public perceptions respectively. The final chapter summarizes the most important issues and choices raised in the previous chapters.

Purdue University AES, 116 Agricultural Administration Bldg., West Lafayette, IN 47907, USA. 181 pp. ISBN 0-931682-28-2. Price: US$10.00

Trade and Development Aspects and Implications of New and Emerging Technologies: The Case of Biotechnology

This report draws on research jointly done by UNCTAD and UNIDO. It provides an overview of the implications of biotechnology on trade and development, especially for developing nations which are dependent on commodity exports.

The report highlights the impact of tissue and cell culture techniques and genetic engineering on the trade and production of commodities such as coffee, sugar, plantains, rubber, vanilla, cocoa, and crops such as wheat, rice, maize. For example, the value of exports of commodities which are likely to be affected within 4-5 years is estimated at US$20.9 billion.

To what extent developing nations will actually *benefit* from the new technology is a moot question. A major part of biotechnology R&D is done by private enterprises in industrialized countries. Intellectual property rights will therefore be a significant factor in the diffusion of new techniques. The possible implications for developing nations of intellectual property rights with respect to trade and development are briefly highlighted in the report.

The report points out that the research objectives pursued in industrialized countries may not be the same in developing countries. Some new techniques, such as diagnostic kits, are directly applicable in developing nations. Most of the techniques generated in and transferred from industrialized countries, however, will have to be adapted to the local conditions. The number of countries that can in fact be expected to be able to use rDNA techniques for crop improvement is estimated at less than ten. Public international research institutes and networks can be instrumental in the improvement of this situation.

Available from: United Nations Conference on Trade and Development [Palais des Nations, CH-1211 Geneva 10, Switzerland], document No. TD/B/C.6/154, 31 p.

Third World Impacts of Biotechnology

The International Labour Office (ILO) has carried out a series of empirical case studies focusing on the Third World impacts of biotechnology. A brief statement of the results by Iftikhar Ahmed of the ILO can be found in *Biotechnology and Development Monitor* (No. 10, March 1992). Details from: Directorate General International Cooperation, Ministry of Foreign Affairs, The Netherlands.

International Directory of Emergency Response Centres

OECD Environment Monograph No. 43
UNEP IE/PAC Technical Report
Series No. 8

The directory is the result of a cooperative project by OECD and UNEP prepared in response to world-wide demand for easily accessible information about sources of help in case of emergencies involving hazardous substances. It contains details of 36 Emergency Response Centres in 29 countries all over the world. The centres have been chosen to meet the following criteria:

- They are accessible to callers world wide, 24 hours a day;
- They respond in the case of accidents involving non-radioactive hazardous substances (some may also respond in case of accidents involving radio-active substances and/or to natural disasters);
- They maintain lists of experts and other information which they will share internationally;
- They serve as co-ordination points for emergency response operations in their countries.

The main purpose of the directory is to help emergency responders in finding immediate advice from foreign experts when an accident involving hazardous substances occurs. By contacting the centres in the directory technical advice may also be obtained in order to help prevent technological accidents and plan and prepare emergency response. It is intended to update and expand the directory regularly.

The *International Directory* is issued free of charge. Copies can be obtained from:

OECD Chemicals Division
2, rue André-Pascal
75775 Paris Cedex 16
Fax: (33-1) 45 24 16 75

or from:

UNEP Industry and Environment Programme
Activity Centre
Tour Mirabeau
39-43 Quai André Citroën
75739 Paris Cedex 15
France
Fax: (33-1) 40 58 88 74

Biotechnology and International Relations: The Political Dimension

This book by Thomas C. Wiegele is a plea for students and professionals in international relations to study the implications of biotechnology. The author argues that the challenges from developments in biotechnology to traditional international relations will be significant and fundamental.

The book covers the international political environment and the meaning of biotechnology. The three core chapters discuss the impact of biotechnology on international law, commerce and war - all major functional areas of concern to international relations specialists. In the last chapter the author elaborates on his plea for social scientists to study the impact of biotechnology.

Several aspects of biotechnology and international law are discussed, such as cross-national environmental effects of biotechnology, national regulatory systems and some international legal instruments. In chapter four, *Biotechnology and International Commerce*, most attention is given to the issue of intellectual property rights. Several problems for developing countries are assessed: privatization of knowledge, the role of international organizations, field testing by companies, the exchange of plant genetic resources, investments and technology transfer. The most interesting chapter turns out to be *Biotechnology and International War* (chapter five). Biological warfare has received renewed attention from the military, as biotechnology greatly enhances the possibilities to carefully engineer "designer" weapons. Thus, international agreements over biological weapons may become obsolete. Not only national authorities, but

also terrorist groups may fall for the appeal of genetically engineered weapons.

The book is a rather short but comprehensive introduction to the subject of biotechnology and international relations.

University of Florida Press, Gainsville [c/o University Presses of Florida, 15 NW 15th Street, Gainsville, FL 32611]. 212 p. ISBN 0-8130-1055-1. Price: £17.67.

Biotechnology Worldwide

Recently COBIOTECH published the book *Biotechnology Worldwide*. It reflects the state of biotechnology in over 50 countries, rich and poor, developed and developing. It includes government policy, national biotechnology programmes, educational initiatives and legislation. Names and addresses of government departments, research centres, national societies and trade associations are provided. The book also contains descriptions of international organizations relating to biotechnology. The national biotechnology profiles were prepared by internal correspondents, who represent their countries in the field of biotechnology.

The book is available at CPS Scientific Limited, Science House, Winchcombe Road, GB-Newbury, Berkshire RG14 5QX.

ICRISAT Groundnut Germplasm Catalogue

The International Corps Research Institute for the Semi-Arid Tropics (ICRISAT) is publishing a Groundnut Germplasm Catalog in two volumes. Volume one will contain passport information and volume two contains evaluation and analysis. The catalogue is a result of the dedicated efforts by a team of multidisciplinary research scientists who have spent almost 15 years in compiling the data that is contained in these volumes.

The catalogue describes the progress made by ICRISAT's Genetic Resources Unit (GRU) in collection and conservation of 12,160 germplasm accessions from 89 countries. These accessions have been characterized, evaluated, and screened for their reaction to different biotic and abiotic stresses that reduce yield. This work has led to the identification of genotypes capable of producing high yields and with good nutritional qualities and sources of tolerance or resistance to various pests and diseases.

Thus, the catalogue presents classified and precise information on the present state of groundnut genetic resources. This information will enhance the capabilities of the international community of scientists involved in the genetic improvement of the groundnut, and will help them select and utilize the most appropriate accessions for use in their national programmes.

Biomaterials: Novel Materials from Biological Sources

Biomaterials is a completely new publication from the Macmillan Press.

This book, written by a team of experts and edited by David Byrom, discusses the subject from both a scientific and commercial perspective while illustrating the requirement for a multidisciplinary approach if successful commercialization is to be achieved. The book is a useful compendium on the nature and production of a wide range of biomaterials such as polysaccharides, hyaluronic acid, alginates, microbial cellulose, collagen, silks and genetics and miscellaneous biomaterials.

The book will be a valuable source of information for anyone involved with biomaterials either at a commercial or non-commercial organization as well as professors of biotechnology-related disciplines.

Available in hardback priced at £50 from Globe Book Services, The Macmillan Press Ltd., Houndmills, Basingstoke RG21 2XS.

Pharmaceuticals, Biotechnology and the Law

Written by a team of experts from Bird & Bird, the book provides a single commentary covering the wide variety of legal and regulatory issues affecting these two industry sectors, reflecting the increasing interest in the value of intellectual property, regulatory development and remedies under competition law. It not only provides full coverage of all aspects relating to pharmaceuticals and the law, but examines the range of legal questions that arise in this area and why the law has developed in a particular way. It deals with the inter-relation of law and regulations in the UK and examines the impact of EC law on UK law and practice. The authors do not presuppose any knowledge of the law, but provide a starting point to anyone, whether scientist, businessman or lawyer, researching or trying to understand the impact of and the background to the law affecting this area.

Available from Macmillan, Globe Book Services, Brunel Road, Basingstoke, Hants RG21 2XS at £85 in hardback.

Biochemical Engineering and Biotechnology Handbook (second edition)

For this new edition each area has been completely updated with the addition of considerable material and six new chapters. In addition, the contents of each chapter are listed in full at the beginning of the

relevant chapter and the index has been significantly extended. A glossary has also been provided since the first edition.

This book could be useful for biological scientists and engineers in the biotechnology, pharmaceutical, brewing, food, dairy, effluent and water treatment and chemical industries. It will be a valued reference tool for all those working in or studying biological and biochemical processes.

Available in hardback priced at £125 from Globe Book Services, The Macmillan Press Ltd., Houndmills, Basingstoke RG21 2XS.

Biotechnology by Open Learning

Among titles published in the Biotechnology by Open Learning (BIOTOL) series, initiated by the Open University of The Netherlands and Thames Polytechnic, are:

- *Biotechnological Innovations in Animal Productivity* which covers such themes as animals in biotechnology, endocrine regulation of the oestrus cycle, manipulation of reproduction, *in vitro* embryo production and manipulation, gene transfer to a whole number, somatotropins in animal production, and vaccines and diagnostics.

- *Energy Sources for Cells* which covers the division of metabolism, methods of studying metabolism, catabolism of lipids, the breakdown of proteins and nucleic acids, the catabolism of organic and man-made chemicals, methylotrophy and methanogenesis, phototrophy and chemotrophy.

- *The Molecular Fabric of Cells* which covers cells, amino acids, proteins, nucleic acids, carbohydrates, lipids, biological membranes and enzymes.

All three books are priced at £19.95 each. Details from: Butterworth-Heinemann Ltd., Linacre House, Jordan Hill, Oxford OX2 8DP or on 0865 310366. Fax: 0865 310898.

Polymers expected to dominate world market by 1996

According to a new market research report by Delphi Associates entitled *Drug Delivery Systems*, polymers are expected to emerge as the leading drug delivery system by 1996, capturing almost 30 per cent of the market with revenues of US$3.3 billion. The majority of total polymer sales now comes from biodegradable polymers, but mucoadhesives, for delivery of thiazide diuretics, and hydrogels and collagens, for delivery of growth factors, are forecasted to fuel growth in polymer revenues as early as 1993. The report is designed to give pharmaceutical executives a comprehensive, strategic overview on the rapidly changing drug delivery market. It is divided into a technical section and a market information section, offers up-to-the-minute detailed analysis of alternative drug delivery systems and presents in-depth future scenarios for the European, US and Japanese markets.

Polymers are presently joined by transdermals and osmotics as the major drug delivery market players, but by 1996 many other systems are expected to emerge including liposomes, monoclonal antibodies, prodrugs, red blood cells, and cyclodextrins. Advances such as the delivery of hormones (LHRH analogues) in biodegradable polymer and nasal systems, transdermal delivery of oestrogen and nicotine, and repackaging of conventional drugs such as Procardia in the OROS osmotic delivery system have prompted greater research opportunities.

The increasing need of pharmaceutical companies to incorporate drug delivery into initial stages of R&D has motivated over 100 groups to begin researching technologies applicable to delivery systems. Biopharmaceuticals therapeutics are one of the areas where drug delivery systems research is most intensive. Approximately 15 biopharmaceutical therapeutics have already been introduced to clinical medicine and more than 100 others are under development.

Companies are also using new delivery systems to extend proprietary positions on drugs that will soon come off patent. The report explains that through repackaging of drugs in alternative delivery forms, companies can maintain an advantage over generic versions of those same drugs.

The world drug delivery systems market is expected to reach US$11.4 billion by 1996. In 1986, this market realized revenues of US$486.8 million and soared to estimated revenues of US$1.8 billion in 1991. Europe accounted for 46.3 per cent of the market in 1991, followed by the US with 43.5 per cent and Japan with 8.4 per cent. Projections are that by 1996 the US will control the world market with an expected 58.2 per cent market share, while forecasts for Europe and Japan are 31.0 per cent and 6.2 per cent, respectively. The report is priced at US$3,500 and available from Delphi Associates, 44 Avenue de Roodebeek, 1040 Brussels, Belgium, Phone: +32(2) 732 5773. Fax: +32(2) 732 6391.

Technical Information for Culture Collections Curators in Developing Countries

The increasing demands on culture collections for authenticated, reliable biological material and associated information has created a need to conserve the microbial gene pool for future study and has highlighted the need for centres of expertise in culture isolation, maintenance,

documentation, identification and taxonomy. To meet the needs of scientists, especially working in the collections in developing countries, the WFCC Education Committee has initiated, with financial support from UNESCO, the publication of Technical Information Sheets (TIS), edited by K. A. Malik, on various collection-related matters. TIS provide guidelines for good practice in culture collections as well as help scientists in the developing countries who are using micro-organisms and cell cultures in their investigations and are usually faced with problems of their safe handling, cultivation, maintenance, conservation, identification, availability, packing and shipping, deposition and ordering of cultures from collections. So far 10 Technical Information Sheets have been published on a few collection related technical matters. In the future it is expected to cover other aspects pertaining to the needs of microbiologists and curators in developing countries. The authors of TIS are international authorities who have described their well practised methods which can easily be performed. The TIS have been distributed free of charge to the scientists in developing countries. In view of the increasing popularity and demand for TIS, UNESCO has provided financial support for the reproduction of all TIS collectively in the form of a booklet. The booklet on *Technical Information for Culture Collections Curators in Developing Countries* is a compilation of all published TIS and contains specialized collective information useful for the scientists and curators of culture collections in developing countries. The methods listed in this booklet can be performed in any laboratory or culture collection regardless of size or economic standing. They have been developed to emphasize that high standards of results can be achieved in laboratories with modest resources and without sophisticated equipment. It is hoped the booklet will prove valuable so that high standards of scientific service can be achieved in laboratories with modest resources and without sophisticated equipment, particularly in the developing countries.

Directory of ASSOBIOTEC

The Italian industrial association for development of biotechnology (ASSOBIOTEC) has produced the 3rd edition of its Directory of Members (in English). In addition to providing general information, such as size, about each of the member companies some details are given of their specific interests/products etc.

Various industrial sectors (such as pharmaceutical, chemical and agro-food) are included.

Details: ASSOBIOTEC, Via Accademia 33, Milano 20131 Italy. Tel.: (39) 26362306; Fax: (39) 26362284/310.

Interpharm's expanded services for health care manufacturers

With over 500 books, regulatory documents, video training programmes and computer software, Interpharm Press claims to be the world's largest single-stop resource for information on health-care technology and regulation.

The new 1992 catalogue contains information on global practices concerning the biotechnology, bulk chemical, diagnostic, medical devices and pharmaceutical industries. Details of the free catalogue from: Meredith Hellestrae, Interpharm Press, 1358 Busch Parkway, Buffalo Grove, IL 60089, USA or on +1(708) 459 8480. Fax: +1(708) 459 6644.

Literature surveys

The Chromatographic Society has introduced literature surveys devoted to different types of separations.

The following surveys are now available:

1. Chiral Separation Survey

Covers from 1985 to 1991 and contains nearly 1,000 entries on papers devoted to chiral separations. The references (which are almost exclusively in English and to the Primary Literature) have been subdivided into various subject groups such as HPLC, GC, TLC, etc., and then arranged in alphabetical order by first author.

2. Supercritical Fluid Chromatography Survey

This survey contains 1,044 references on papers devoted to supercritical fluid chromatography.

3. Capillary Electrophoresis Survey

The survey covers a period up to May 1991, and has over 580 entries covering many sources on CE.

All the above surveys are available for purchase by members and non-members. Further details available

from: The Secretariat, The Chromatographic Society, Nottingham Polytechnic, Burton Street, Nottingham NG1 4BU. Tel.: 0602 500596. Fax: 0602 500614

BioTech ALERTS

BioTech ALERTS, a new current awareness service based on the CAB Abstracts database, cover the world's literature in agricultural biotechnology and related subject areas. Output is provided on a monthly basis on floppy disk, in comma-delimited format. Other floppy disk products from CAB cover gene mapping and gene transfer, biotechnology and livestock improvement, cloning and gene sequencing, genetic engineering applications, waste and organic by-product processing, and diet, feedstuffs and performance regulators. Details from: CAB International, Wallingford, Oxon OX10 8DE, UK.

ICGEBnet computer resource for molecular biology

Efficient storage and retrieval of protein and DNA structural data such as sequences and 3D structures is one of the central problems of molecular biology. ICGEBnet is a computer resource maintained at the International Centre for Genetic Engineering and Biotechnology, Trieste, that can be accessed through remote login from 45 countries. The service includes on-line access to databases through an international computer link, bulletin boards, electronic mail and a host of freely available PC software. ICGEB is part of EMBnet, the informatics network of the European Molecular Biology Organization. The major databanks available on ICGEBnet are:

1. EMBL 29: EMBL nucleic acid sequence data bank;
2. EMBL-Daily: EMBL Daily update [through EMBnet];
3. GenBank 70: NIH nucleic acid sequence data bank;
4. PIR 31: Protein Identification Resource protein database;
5. Swiss-Prot 20: EMBL/University of Geneva protein database;
6. SEQDB 91.9: PRFOsaka Peptide/ Protein Sequence Database;
7. SBASE 1.0: ICGEB-Trieste Protein Domain Library;
8. Enzyme 9202: Restriction Enzyme Data Bank;
9. HIV-NA/AA 1: HIV nucleic acid and protein database;
10. Prosite: EMBL/University of Geneva protein pattern database;
11. PIsearch: Protein consensus sequence-pattern database;
12. PBASE: Collection of amino acid residue physical parameters;
13. Bibliography: Molecular biology computer applications.

Sequence analysis is provided by the IntelliGenetics Suite of programs, as well as FastA [similarity search], Phylip [sequence phylogeny analysis], CLUSTAL [multiple alignment] and a host of other programs installed on the SUN 4/390 computer.

ICGEBnet specializes in search methods for distant protein sequence homologies. ICGEB maintains SBASE, a library of protein domains with over 24,000 entries and is developing methods based on parametric representation and Fourier analysis.

For further information contact: Dr. Sándor Pongor, ICGEB, Padriciano 99, 34102 Trieste, Italy. Tel.: +39-40-37571. Fax: +39-40-226555. Tlx: 460396 ICGEBT I. Email: pongor @ icgeb.trieste.it. (Source: *Helix*, May, 1992)

Two new biotechnology PC database systems

In response to customer demand, Abstracts in BioCommerce (ABC), the well known biotechnology business news monitoring service published since 1982 as a twice-monthly journal and online database is being made available to subscribers on floppy disks. Complete with simple, function-key driven software suitable for IBM compatible personal computers, PC-ABC will be issued every two weeks on 1.44MB 3.5 inch disks with quarterly and annual files supplied monthly. Backfiles covering the period 1981-1991 will also be available.

Since 1981 ABC has summarized over 250,000 news reports, using a unique multicitation abstracting system to remove duplication while providing full bibliographic details of all the articles describing an event. ABC is now supported by an extensive document delivery service, enabling users to obtain copyright cleared photocopies of most cited sources, even those no longer in print.

In addition, a complete database version of Biotech Knowledge Sources (BKS) is available exclusively on the MSDN network. BKS is a monthly service listing new publications on all aspects of biotechnology and a comprehensive conference calendar. It covers recent books, new periodicals, market research reports, videos, databases, directories and "grey literature" such as government documents, and culture collection catalogues as well as meetings training courses

and exhibitions world wide with the emphasis on US and European venues. The BKS file on MSDN contains over 2,200 entries and will be updated monthly.

For further information on either products or to obtain demonstration disks, contact Customer Services, BioCommerce Data Ltd., Prudential Buildings, 95 High Street, Slough, Berks., SL1 1DH, UK. Tel.: (0753) (Int +44 753) 511777. Fax: (0753) (Int +44753) 512239.

BioCommerce Data is a specialist publishing and consultancy company, producing the biotechnology business information database, BioCommerce Abstracts and Directory, available through Dialog and Data-Star. Its printed publications include Abstracts in BioCommerce (a twice monthly news index), Biotech Knowledge Sources (a monthly bulletin listing new publications and forthcoming conferences) and the UK Biotechnology Handbook 91/92 (a directory of British organizations). The company also provides a mailing list rental service based on its international directory database which enables top executives in the biotechnology industry world wide to be selectively targeted for direct mail promotions.

Vital scientific software simplifies protein quantification studies

Accurate comparison between electrophoresis gels can be greatly simplified using Quantity One software running on a PDI DeskTop scanning densitometer (configured with a Sun Microsystems SPARCStation). This new approach should have a major impact on protein and DNA quantitation studies. By allowing any number of bands on different gels to be selected for comparison, the software allows the maximum data to be obtained from a gel, autorad or blot. Details from: Vital Scientific Ltd., Huffwood Trading Estate, Partridge Green, Sussex RH13 8AU or on 0403 710479.

Biodegradation journal

A new journal, *Biodegradation*, will publish papers on all aspects of science relating to the detoxification, recycling, amelioration or treatment of waste materials and pollutants by naturally-occurring microbial strains or associations of recombinant organisms. Details from: Kluwer Academic Publishers Group, Order Dept., P.O. Box 322, 3300 AH Dordrecht, The Netherlands or on +31 78 524400. Fax: +31 78 524474.

G. SPECIAL ARTICLE

POLYMERASE CHAIN REACTION AND ITS APPLICATIONS

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Introduction

Advances in recombinant DNA technology during the past two decades have had a profound impact on current biological research and medical applications. From the early 1970s to the mid-1980s, a series of discoveries resulted in rapid technical advancements in nucleic acid characterization, molecular cloning, sequence determination and the use of nucleic acid probe-based tests in clinical fields. These discoveries included prokaryotic modification, restriction systems and restriction endonucleases.

The recent development of the polymerase chain reaction (PCR) in 1985 by Mullins and his co-workers (100, 131) further accelerated the advancement of molecular technologies and broadened their applications. PCR is currently being utilized to assist biomedical research, identify and detect infectious agents and genetic defects, study molecular evolution and to conduct medical epidemiology and forensic medicine. Because PCR made these dramatic advances in all fields of biological sciences possible, it was named "molecule of the year" (1989) by the journal *Science*. The biotechnology industry expects PCR to sweep the clinical diagnostics market in the next few years and replace many current modes of testing. An impact on environmental monitoring is also anticipated.

PCR and its applications in various fields have been discussed in many recent reviews. (13, 15, 45, 109, 117, 161) This article describes standard and inverse PCR techniques. The potential for PCR in the diagnosis of diseases is considered and in particular, reference to tropical diseases in developing countries is emphasized. Other applications for forensic medicine and detection of genetic disorders are also discussed.

Principle of PCR technique

The impact of PCR on the biological sciences is enormous but the principle of PCR is remarkably simple. During this *in vitro* procedure, the target DNA undergoes an exponential replication of up to several million fold, even in the presence of a vast quantity of unrelated DNA molecules. The resultant highly homogeneous DNA becomes an excellent source for diverse molecular manipulations. As shown in figure 1, PCR requires a three-step cycling process: (a) denaturation of double stranded DNA templates, (b) annealing of short oligonucleotide primers with the templates, and (c) synthesis of new copies of the template sequences by DNA polymerase. A cycle, conducted in a reaction vessel, generally takes 3 to 5 minutes and is repeated 30 to 40 times. The entire process takes 2-4 hours to complete. Modifications of the reaction vessels and the temperature-controlling device have been made recently to reduce the total amplification time to less than 30 minutes and to increase the amplification specificity. (160)

PCR is carried out in a small volume, typically 100 µl in a 0.5 ml microfuge tube. It can also be performed in a small glass capillary tube (159, 160) where heat transmission is highly efficient. The reaction generally contains four deoxynucleoside triphosphates (200 µM each), specimen DNA, 10 mM Tris HCl buffer, pH 8.3, 2 mM $MgCl_2$, 50 nM KCl, 100 nM each of two oligonucleotide primers, and 2.5 units of *Taq* polymerase. The duplex formation between the primers and target DNA is both sequence and temperature dependent. Therefore, to achieve an optimal amplification, (156) it is advisable to empirically determine the molarity of each chemical ingredient, the amount of DNA polymerase, the temperature and the number of cycles.

DNA polymerases

The original PCR reaction was directed by the DNA Klenow fragment of the *Escherichia coli* DNA polymerase. The Klenow fragment is heat-labile and is destroyed at the 95°C temperature required for DNA denaturation, necessitating the addition of fresh enzyme after every denaturation step. The use of a thermostable DNA polymerase isolated from various thermophilic micro-organisms avoids this shortcoming. The first thermostable DNA polymerase was the 110 kDa *Taq* polymerase purified to near homogeneity more than two decades ago. It was isolated from *Thermus aquaticus* recovered from a hot spring in Yellowstone National Park. (18) *Taq* has a half life at 95°C of approximately 40 minutes (49) and because of this thermo- stability, the DNA annealing and synthesis steps of PCR can be conducted at a temperature near the melting temperature (Tm) of the primer/DNA template duplex. The higher temperature eliminates non-specific matching between the primer and non-target sequences, which results in extraneous amplified products.

Stoffel fragment, (142) a 61 kDa fragment of the *Taq* enzyme, has even greater thermal stability than its parent *Taq* enzyme and is suitable for the amplification of G+C rich DNA templates. It also works at a broad range (2-10 mM) of magnesium ion concentration. This is particularly useful when performing multiplex PCR (27, 149) with the simultaneous amplification of two or more targets in the same reaction. Many more thermostable DNA polymerases have been purified recently. (22, 23) *Vent* and *Tth* polymerase are derived from the bacteria *Thermococcus litoralis* (23) and *Thermus thermophilus*. (22) respectively. A thermostable DNA polymerase is also purified from *Archaebacterium sulforolobus* (132) and is shown to be useful in PCR. These new thermostable DNA polymerases may have some advantages over the original *Taq* polymerase. *Taq* DNA polymerase lacks 3' to 5' exonuclease activity, a "proofreading" or "editing" function essential for the fidelity of the DNA reproduction process. Thus, in the presence of high concentrations of deoxynucleoside triphosphates (dNTPs) and the divalent ion, $MgCl_2$, the misincorporation rate of nucleotides into the growing DNA chain can be as high as 10^{-4} per reaction cycle. (49) This misincorporation rate can be reduced tenfold or more by reducing the concentrations of dNTPs and $MgCl_2$. (49) *Vent* polymerase possesses the proofreading activity and can operate at a wider range of dNTP and $MgCl_2$ concentrations than *Taq* polymerase. *Vent* also has a longer half life. (23)

The *Tth* enzyme, on the other hand, is unique. In addition to the DNA-dependent DNA polymerase activity, *Tth* possesses an RNA-dependent DNA polymerase, or reverse transcriptase activity. (101) A significant problem in using RNA as a template is the inability of standard reverse transcriptases, derived from murine or avian retroviruses, to synthesize cDNA from certain RNA regions that are G+C rich or contain complex secondary structure. With the *Tth* polymerase, the reverse transcription step can be performed at a temperature high enough to release the secondary structure of RNA. The *Tth* polymerase has been shown to be considerably more efficient than *Taq* polymerase and the sensitivity of detection for PCR is greatly enhanced. (145) In addition to the convenience of *Tth* polymerase in performing both cDNA synthesis and PCR amplification, the high degree of specificity and sensitivity of the enzyme should enable scientists to find wide applications in diagnostics and molecular biology.

Source of target specimens

RNA or DNA from many sources can be targeted for amplification. For biomedical research and clinical diagnostic assays, the most common sources of specimens are blood, body fluids and fixed tissues. (33, 37, 42, 53, 67, 72, 95, 124) Because of the exquisite sensitivity of the PCR assay, only a small amount of specimen is necessary. For example, genomic DNA derived from a single hair follicle can be used. (59) The DNA specimen does not need to be purified and in many cases, whole

blood can be used directly. (97, 115) The *Taq* polymerase can function in harsh conditions where detergents (155) and small peptides are present. Hence, the targeted specimens can be prepared using detergents (Nonidet P40, Tween 20 and/or Triton X 100) or chelating agents to disrupt the cell membrane, (152) and a protease (e.g., proteinase K) to digest cellular proteins. It is generally unnecessary to purify DNA using organic solvents like phenol and chloroform, although some organic solvents are reported to increase the amplification efficiency. (121) Blood or deparaffinized tissue can also be boiled in a small aliquot of distilled water and the supernatant can be used directly for PCR amplification. It has been reported that a single 5 μm section of paraffinized tissue, prepared by using nonionic detergents and protease, is sufficient for approximately 20 PCR reactions. (150) Gene amplification from Guthrie blood spots, which have been used in infant metabolic screening, is also successfully achieved. (24, 92, 102) Thus, PCR enables the use of numerous histologic, pathologic and metabolic archival materials for retrospective archaeological studies at the molecular genetic level.

Primer selection and reaction components

The primers are single stranded DNA oligonucleotides of approximately 18 to 40 bases in length. Primers specifically recognize the target DNA by their sequence complementarity and act as the initiation points for new templates of DNA synthesis. The base composition, usually 50-60 per cent G+C content, together with the nucleotide sequence, critically determine the strength of the DNA duplex formed by the primer and target DNA. Many computer software programs are designed to help select appropriate primers for amplification. (60, 129) A DNA duplex with a high Tm has a greater advantage over a weak one, since the amplification can be carried out at a high temperature to reduce non-specific pairing.

The bases at the 3' end of the primer sequence play the most critical role in the initiation of DNA elongation. A single nucleotide mismatch at this end greatly reduces the priming efficiency. After 30 rounds of amplification, the amount of amplified product primed by a primer with a single mismatch can be only a small fraction (less than 1 per cent) of that primed by perfectly matched primers. (77) On the other hand, this property has been adapted, as shown in the amplification refractory mutation systems (ARMS), (103) to detect defined point mutations present in genetic defects, or variants of infectious agents or drug resistance (4, 79, 80, 124) in micro-organisms.

The complexity of the human genome is approximately 3×10^9 base pairs. A primer sequence could, by chance, have homologous but not identical counterparts in the human genome and result in non-specific amplification. Therefore it is important to empirically test the specificity of the primers prior to routine use. On the other hand, primers with some degree of degeneracy can be used to fish out their related but previously uncharacterized sequences from the human genome. (90)

Detection of amplified products and oligonucleotide probes

Amplified DNA can be detected either by direct visualization after gel electrophoresis and ethidium bromide staining, or by hybridization with specific DNA probes. Advantages and disadvantages of gel analysis and two hybridization methods are shown in table 1. Direct gel visualization is simple: amplified DNA products are loaded directly onto an agarose or a polyacrylamide gel for electrophoretic separation. Specimen DNA is compared to DNA of known size, which is visible after ethidium bromide staining. This approach, however, suffers two major drawbacks. First, it is not sensitive since many positive specimens may have amplified DNA product but are of insufficient quantity for direct visualization. Second, it lacks sequence specificity; and non-specific DNA can be amplified which has an electrophoretic mobility similar to that of a specific amplified product. Therefore this detection procedure alone is not recommended for routine clinical use. Southern blot analysis overcomes these drawbacks. The DNA in the gel is transferred onto a solid support (e.g., nitrocellulose or nylon filters) and target-specific probes are used to hybridize with the amplified DNA fragments. Southern blot analysis thus provides a higher sensitivity and specificity compared with direct visualization on gels. However, the procedure is time-consuming and labour-intensive.

In solution hybridization, amplified DNA and probe are mixed in a hybridization solution of an appropriate NaCl concentration. The mixture is denatured at 95°C for 3-5 minutes to dissociate the amplified double-stranded DNA and is then cooled to 50-60°C to permit the hybridization between the probe and its complementary sequence to occur. The hybridization mixture is electrophoresed in a polyacrylamide gel to separate the hybridized (slow-moving) and the free (fast-moving) probes. If the probe is radiolabelled, the gel can be exposed to X-ray film for a few hours at -70°C. The band of hybridized DNA can then be visualized. This procedure is often referred to as oligomer hybridization.

Recently, it has been reported that 5' to 3' exonuclease activity could be employed to detect amplified DNA and generate a specific detectable signal. In addition to the two primers used in PCR, an oligonucleotide probe, isotopically labelled at the 5' end, is included in the assay. This DNA probe has a non-extendable 3' end and cannot be used as a primer for extension, but it can hybridize with amplified DNA product. During amplification, the 5' to 3' exonuclease activity of the *Taq* polymerase degrades the hybridized

DNA probe. A positive sample is indicated by the reduction in the size of the isotopically labelled DNA probe. This new assay (61) represents a significant improvement over many previous cumbersome isotopic detection methods. Because the amplification and detection reactions are simultaneously performed in the same reaction vessel, the chances of PCR-related contamination problems, as discussed below, can be eliminated. However, this newly described assay is still based on isotope-labelled probes and therefore suffers many drawbacks inherent to isotopic tests. Substitution of the isotopes with other nonisotopic detectors would be useful.

Since PCR is going to be routinely used in clinical laboratories for numerous genetic diseases and infectious agents, it is advantageous to use non-isotopic detecting probes. For example, probes labelled with biotin (47, 70) or digoxigenin (85) are easy to prepare and have a long shelf life. Another variant of solution hybridization is the hybridization protection assay or homogeneous protection assay (HPA) using chemiluminescent DNA probes. (111) The advantages of this method are: (a) the reaction is simple because there is no need to physically separate the hybridized and unhybridized probes; (b) the reaction time is short, usually less than one hour; (c) the read-out is digital making it very easy for a laboratory researcher to judge and record results; and (d) the test is quantitative in nature, thereby enabling the estimation of the amount of infectious agents in the original specimens. (71)

Errors associated with PCR and remedies

Since PCR is so sensitive that it can detect just a few target molecules, it is also highly susceptible to contamination. The contamination or carry-over of a few molecules of previously amplified DNA into a specimen to be tested can inadvertently result in false positivity. (31) A laboratory repeatedly performing PCR is vulnerable to false-positive results due to the accumulation of amplified product in the workplace. Various physical, chemical and enzymatic measures are recommended to reduce contamination problems: (a) single-use reagents; (b) different rooms for DNA specimen preparation, PCR amplification and post-PCR detection; (c) positive-displacement pipetting devices and (d) the use of UV- irradiation, (113, 133) isopsoralen, (30) exonuclease III, (165) gamma irradiation (42) or restriction endonucleases (41) to inactivate or destroy contaminating DNA.

Longo et al. recently developed a method using uracil DNA glycosylase (UDG) and deoxyuracil triphosphate (dUTP) to control carry-over contamination. (87) This new reaction contains dUTP instead of deoxythymidine triphosphate (TTP) so that the *in vitro* synthesized DNA contains uracil bases instead of the thymine bases present in the natural DNA templates. UDG enzyme degrades uracil-containing templates at 37° C but does not affect the natural thymine-containing DNA. UDG enzyme is included in the PCR reaction mixture. If contaminating DNA molecules are carried over to a new DNA specimen, they will be degraded by UDG in the beginning of the PCR reaction. UDG is inactivated at 95° C, the temperature required for the first annealing step of PCR, and thus will not destroy the newly synthesized DNA during PCR.

Other related techniques

Several new techniques have also been developed to amplify target DNA sequences to allow for molecular manipulations. These techniques include (a) self substantiated sequence replication (SSSR or 3SR), (55) (b) ligase chain reaction (LCR) (9) and (c) Q-beta replicase mediated RNA amplification. (75)

The 3SR system uses reverse transcriptase, RNase H, and RNA polymerase enzymes to produce numerous RNA molecules at a constant temperature (around 37 to 40° C). This technique can be applied to DNA or RNA templates. If the starting material is double stranded DNA, a heat-denaturing step at a high temperature (95° C) is necessary, as in PCR. If the starting material is RNA without complex secondary structure, the high temperature denaturation step is not required. One of the primers has a composite sequence, which includes an RNA polymerase promoter sequence at its left side (i. e., 5' end) and a sequence specific to the target DNA or RNA at its right side. When this primer is extended, the resultant new DNA can be copied by the RNA polymerase to produce several hundred copies of RNA transcripts in a single cycle. 3SR has been used to detect HIV-1 proviral DNA and AZT resistant HIV-1 in the lymphocytes of HIV-infected persons. (55, 124) If the amount of a target RNA is to be measured from a specimen also containing target DNA, 3SR appears to be the method of choice. In PCR, it is necessary to remove DNA using DNase before initiating the reverse transcription step. By using 3SR, the reaction can be started at a low temperature, at which the DNA template would not be copied. The subsequent step, the RNA polymerase reaction, would generate RNA copies only from the DNA copies containing the RNA polymerase promoter sequence and the specific target DNA sequence, but not from the original target DNA. The use of 3SR to aid in the evaluation of theraputic treatment of infectious diseases (especially RNA viruses) is anticipated.

LCR employs two sets of adjacent oligonucleotides. The thermostable ligase covalently joins each set of the oligonucleotides if there is perfect complementarity at the junction. (8, 9) LCR creates a double stranded DNA product composed of four oligonucleotides without DNA synthesis. The double stranded, ligated DNA product can then serve as another template to hybridize with unligated DNA oligomers. The hybridization and ligation process can proceed in a chain reaction manner as does PCR with DNA replication. The ligation reaction is base-specific, if there is a mismatch at the 3' end of an

oligomer which is to be linked to the 5' end of the next oligomer, no ligation will occur. (8,9) Thus, LCR can be used to detect point mutations by using a set of four oligomers with different ending nucleotides. It is now being used successfully to detect point mutations in sickle cell anaemia. (8,9) LCR can also be linked to PCR or 3SR as a detection assay. (8) It has great potential in detecting a vast number of point mutations in genetic disorders and infectious pathogens.

Use of PCR in identifying unknown sequences adjacent to a known sequence

Regular PCR employing a primer pair of known sequences allows the amplification of a DNA segment bounded by the two primers. However, this process does not permit the acquisition of sequence information outside the boundary defined by the two primers. The sequence information in the region flanking a known sequence is of particular importance in studies of chromosome walking (134) and of the integration sites of viruses or phage in chromosomal DNA.

Several modifications of the regular PCR have been made in the inverted PCR (IPCR) procedure to allow the amplification of DNA flanking a known DNA sequence. (136) Instead of having the two primers facing each other as in the regular PCR, the two primers in the IPCR are facing outwards, as shown in figure 2. The target DNA has to be digested with a restriction endonuclease(s) or sheared by physical means to generate a linear DNA. The linearized DNA is then circularized by DNA ligase through an intramolecular ligation event. The two primers hybridized with the circular DNA now face each other and flank the unknown sequence. Using this inversion process, the unknown sequence flanking a known sequence can be readily amplified and analysed. Limitations of IPCR include the use of restriction endonucleases and the potential formation of a hybrid DNA through intermolecular ligation events. Thus, it is important to perform Southern analysis to ensure the physical linkage of newly obtained sequences with the known sequence. Silver et al. recently reported the use of IPCR to determine the cellular DNA sequence adjacent to an integrated murine leukaemia proviral DNA without a labour-intensive molecular cloning process. (137)

To eliminate the need of knowing the two DNA primer sequences and the location of restriction endonuclease sites within a reasonable distance, new procedures have been developed. (35, 48, 86, 88) When the unknown sequence is near the 3' end of a messenger RNA, oligo-dT can be used as one of the two DNA primers. Shyamala and Ames (136) reported a procedure using a cloning vector (e.g., M13 phage DNA) to facilitate chromosome or genome walking. The cloning vector not only provides a means to clone and propagate the target DNA, but also provides a primer site in the recombinant molecule. Since this procedure requires the knowledge of only a single primer specific for the target DNA, it is called single-specific primer PCR or SSP-PCR. Amplification can be performed directly in the ligated DNA mixture to generate double stranded DNA molecules. The amplified DNA product can be sequenced to yield DNA fragments of 300-400 nucleotides immediately adjoining the primer sequence. From the newly acquired fragment, a new primer oligonucleotide is then used to repeat the amplification and sequencing process. This process does not require the growth and selection of phage containing the target DNA. However, if the isolation of the recombinant phage is necessary, it can be easily conducted using the same ligation mixture to transform a suitable bacterial host for phage propagation and selection by appropriate DNA probes. Thus, SSP-PCR is ideal for the amplification of genomic DNA for intron-exon junctions and progressive and unidirectional genome walking into an unknown region. (136, 134)

PCR in forensic medicine

DNA fingerprinting (65) has become the major means in forensic medicine to include or, more importantly, to exclude a person suspected of committing a crime. Paternity and HLA (histocompatibility antigens) tests are likewise facilitated by PCR. In many cases, the only available materials at crime scenes are small amounts of blood, a few hairs or a small volume of semen. In order to obtain sufficient information from such a small amount of specimen containing DNA, researchers use Variable Numbers of Tandem Repeat (VNTR) loci. (65) VNTRs are stretches of DNA in which a short nucleotide sequence is repeated 20 to 100 times consecutively. Different VNTR alleles are composed of different numbers of repeats containing changes which vary significantly from person to person, unless they are genetically identical, such as identical twins or genetically closely related. (65)

DNA fingerprinting requires 50 ng (or 7,500 cell equivalents) to 1 µg (150,000 cell equivalents) DNA. By using PCR, it becomes feasible to use only a few white blood cells, hair or a small amount of sperm as the starting material. (59, 83, 84, 126, 156) Partially degraded DNA specimens can also be used. Extensive comparison between the sequences derived from a suspect and the specimens collected at a crime scene can be made in a statistically sound manner. More than 2,000 DNA fingerprinting tests have been submitted to US courts. (146) However, the validity of calculating the probability of finding a match in DNA type among a given population is currently under intensive debate. (26, 82)

Diagnostic applications in paediatric HIV infection

Of all the infectious agents plaguing mankind, the proviral sequence of human immunodeficiency virus (HIV) present in peripheral blood lymphocytes of HIV-infected patients was the first shown to be detectable by means of PCR. (112) In HIV-infected

persons, the level of virus load in circulating lymphocytes is extremely low, usually one proviral copy in a few hundred, to one in a few thousand lymphocytes. (135, 138) Approximately 20-30 per cent of infants born to seropositive mothers are infected with HIV either *in utero*, during birth or through breast-feeding. (114) Generally, the infection status of an HIV-infected person is determined by the presence of antibodies, antigens or culturable viruses in the blood. Unfortunately, the presence of maternal IgG antibodies which can persist for up to 15 months, the poor sensitivity of antigen assays and time-consuming and labour-intensive culture assays make early diagnosis of HIV infected infants extremely difficult. Rogers et al. (127) as well as others (46) have reported that PCR could be used to correctly identify most infected infants within the first 2 to 3 months after birth. Infants who seroreverted, for example, lost their maternal antibodies and did not develop their own antibodies against HIV, were not truly infected and were also PCR-negative. Other paediatric tests including antibody-producing cell assays, (81) improved antigen assays and IgA tests (122, 153) are being extensively evaluated. (36) A few studies suggest that IgA assays are able to detect 6-10 per cent of infected infants at the age of one month, 57-67 per cent at three months and 77-94 per cent at six months (122, 153). Detection of IgA has the advantage of being simple to perform, providing rapid results, having a lower cost and being easily adaptable for commercial use; (127) but PCR appears to give the earliest diagnostic determination. (46, 127). PCR identifies many HIV-infected newborns in the absence of any clinical symptoms or other laboratory evidence of infection. (127) This is important because early intervention using anti-retroviral therapy in newborns should be implemented as soon as possible before immunodeficiency and symptomatic illness are manifest.

PCR applications in genetic diseases

Development of reliable tests in medical genetics for children at risk of disorders such as cystic fibrosis (CF), Duchenne muscular dystrophy (DMD), and haemoglobinopathies has been under intense pursuit for many years. The application of DNA probing techniques to diagnose haemoglobin disorders is performed at 8 to 10 weeks of gestation using DNA extracted from chorionic villi. (108) Three strategies were used to detect genetic defects involving point mutations or deletions: (a) if a mutation alters a restriction endonuclease cleavage site, the failure of restriction cleavage indicates the presence of a mutation; (b) the use of specific oligonucleotide probes to differentiate wild type and mutated sequences; (144) and (c) the use of restriction fragment length polymorphorphisms to identify the change of an inherited pattern through pedigrees. (19) These approaches, like those used in forensic investigations, require a sizeable amount of DNA and are time-consuming and cost-prohibitive. The recent use of PCR in the field of genetic disorders in conjunction with the newly acquired knowledge of the gene sequences of many genetic disorders, facilitates the advancement of prenatal diagnosis of genetic defects.

CF is one of the most common inherited disorders, affecting about one in 2,000 children. The CF gene was discovered in 1989 and is referred to as the CF transmembrane conductance receptor. Approximately 70 per cent of the mutations in CF patients correspond to a specific deletion of three base pairs at amino acid position 508 (delta F_{508}) of the CF gene. (7) Allele-specific oligonucleotide primers corresponding to the wild type and the delta F_{508} alleles can be used in PCR to identify persons carrying homozygous or heterozygous mutated CF genes. (7) Thus, it is possible to carry out prenatal diagnosis and carrier screening for most, but not all of the affected foetuses and CF carriers. However, whether the prenatal diagnosis and carrier screening programme are scientifically and socially justified remains to be answered. (39)

Analysis of mutations at the DMD locus is another example in which an enormous gene and transcript necessitates PCR amplification of many large regions of DNA. Like CF, DMD is one of the most common human genetic diseases and affects approximately one in 3,500 male births. One third of all DMD cases arises via new mutations. (50) The muscle distrophin gene is greater than 2 million base pairs in size and contains at least 70 exons separated by an average intron size of 35 kbp. Partial intragenic deletions account for up to 60 per cent of all cases of this disease. Therefore it is important to co-amplify as many of the mutations as possible in a single assay using multiplex PCR. (27) Multiplex PCR has been shown to identify 80 to 90 per cent of all dystrophin gene deletions. (27)

PCR application in microbiology and in developing countries

Routine clinical microbiological diagnosis employs numerous cultural, biochemical and enzymatic methods to differentiate and characterize the micro-organisms present in a given specimen. Many routine bacterial diagnostic tests are so well-established that they are not expected to change significantly in the near future. However, several specific areas of microbial detection can be greatly improved by PCR technology. These areas include: (a) the micro-organisms which are difficult or expensive to culture, for example, viruses, spirochetes, chlamydia, mycoplasma, anaerobic bacteria and mycobacteria; (b) pathogenic bacteria possessing toxins which are difficult to detect; (c) antimicrobial drug resistant bacteria (4) and AZT resistant HIV-1 strains; (79, 80, 124) (d) micro-organisms that do not survive the transfer to culture medium; (e) species differentiation within a bacterial genus; and (f) cross-species amplification of both prokaryotic and eukaryotic organisms. Examples of the use of PCR to identify many pathogenic micro-organisms of medical

importance are listed in table 2. It is important to establish the clinical relevance of a positive PCR test for a micro-organism since a positive PCR test may not correlate directly with clinical manifestations. If properly performed, PCR can be used to quantify the amount of the micro-organisms in a given specimen. The future use of PCR in this manner to correlate the amount of micro-organisms with clinical manifestation is anticipated.

The eubacterial ribosomal RNA genes (16s and 23s) contain some segments that are conserved at the species, genus, or kingdom level. (96, 158) Based on sequence variations in the ribosomal genes, Barry et al. recently used specific PCR primer pairs and probes to differentiate *Clostridium perfrigens* from other organisms in the genus. (10) Similarly, Riley et al. using a singie set of T17 primer sequences was able to amplify DNA products to distinguish different isolates of *T. vaginalis* (125) based on the electrophoretic properties of the DNA products. Sequences closely related to the T17 primers are also present in many eukaryotic micro-organisms in a species-specific manner. Thus the same PCR primer pair can also yield distinguishable patterns of amplified products from pathogenic protozoan parasites, including *Giardia lamblia, Leishmania donovani, Trypanosoma* and *Acanthamoeba*; the nonpathogenic protozoans, *Paramecium tetraurelia* and *Tetrahymena thermophilia*; and a yeast, *Saccharomyces cerevisiae*. This approach holds promise as a tool for the identification of organisms at the species level and for the development of new methods to clinically diagnose or identify new non-culturable bacterial pathogens. (158)

Many infectious organisms prevail in developing countries located in tropical and subtropical regions of the world. Micro-organisms like *C. trachomatis, M. tuberculosis*, Epstein-Barr viruses, Hepatitis B and C viruses. Dengue viruses, and human immunodeficiency viruses (HIV), are difficult to culture and detect. Bobo et al. evaluated the utility of PCR in the diagnosis of *C. trachomatis* in ocular specimens from persons living in a trachoma-endemic area of Tanzania. (16) They found that PCR is more sensitive than the direct antibody immunofluorescence assay currently used in the area. (16) PCR has been shown to successfully detect Hepatitis B and C viruses, (29) and Dengue viruses (99) and subtypes of influenza viruses (28, 162) in many developing countries. Enteroviruses, the most common viruses in contaminated water, are responsible for many outbreaks of diseases in developing countries. Jothikumar et al. conducted water quality surveillance programmes in India by using PCR to detect enteroviruses in the water supply. (68) The use of PCR in detecting some of the medically important pathogens in tropical and subtropical countries is listed in table 2. To optimize the utilization of PCR in these developing countries, it is crucial to meet the following requirements: (a) the collection, preservation and transport of specimens should be simple; (b) if the test requires detection probes, non-radioactive detection probes should be developed; (c) the cost should be affordable; and (d) the PCR test should have an obvious clinical advantage over current laboratory detection methods.

PCR applications in environmental sciences

The applications of PCR in environmental and ecological monitoring of micro-organisms is anticipated to dramatically increase in the next decade. The detection of coliform bacteria, *Escherichia coli* in particular, in various water sources is one example. Water quality is reflected by the level of coliform bacteria derived from human and animal faecal material. Traditionally, coliform bacteria are cultured, then identified according to their specific nutrient utilization patterns. These tests take several days to complete, but using the new PCR techniques, particularly multiplex PCR, several pathogens can be simultaneously detected in just a day or two. (11) Similarly, PCR offers a rapid method for monitoring enteric bacterial and viral pathogens including enteroviruses, *Salmonella* and *Shigella spp*, and pathogenic protozoa such as *Giardia* and *Entamoeba*, in various agricultural and water sources. *Legionella pneumophila*, which causes legionellosis, is widely distributed in water supplies, cooling towers and air-conditioners. *L. pneumophila* and other legionella in environmental water sources can now be detected on the basis of their respective 5S ribosomal RNA gene sequences. (2)

Conclusion

The progress of PCR as a molecular tool in facilitating medical research during the past few years has been remarkably rapid. Over the next few years, in conjunction with other molecular techniques, PCR will continue to expeditiously expand our acquisition of genetic markers for human genes and many medically important infectious pathogens. Utilization of such genetic markers will contribute to the rapid elaboration of human genetic maps, and to the development of other markers for health related traits in medicine. Extensive applications in population genetics, agricultural sciences, fishery sciences (57) and environmental and ecological surveillance (2) are also anticipated.

Table 1

Detection methods for amplified DNA products

Method	Advantages	Disadvantages
Direct gel anal	Fast (30 minutes) Size is known	Insensitive Not specific
Southern blot analysis	Specific Size is known	Tedious Time consuming
Solution hybridization	Fast (1-5 hours*) Sensitive Specific	Size unknown

* Chemiluminescent probes can detect DNA product in 1 hour, whereas biotinylated or isotopic probes require 4-5 hours or longer.

Table 2

Diagnostic detection of selected pathogenic micro-organisms of medical importance by PCR

Viruses
- HIV (6, 112, 135, 138)
- HTLV (25, 40, 93)
- Hepatitis virus A (64)
  B (62, 73, 92)
  C (38, 107)
- Herpes simplex virus (5, 74, 105, 118)
- Papilloma virus (91, 141, 148, 147, 157)
- HHV-6 (34)
- Epstein-Barr virus (76, 143)
- Rubella (43)
- Rabies (130, 140)
- Cytomegalovirus (20, 52)
- Rotavirus (44, 51)
- Adenovirus (1)
- Lassa virus (89)
- Coxsackie virus (154)
- Influenza (28, 162)
- Dengue (99)
- Poliomyelitis virus (163)

Protozoa, Rickettsia and Bacteria
- *Toxoplasma gondii* (3, 66)
- *Trichomonas vaginalis* (10)
- *Pneumocystis carinii* (151)
- *Plasmodium falciparum* (63)
- *Clamydia trachomatis* (58, 104, 110, 120)
- *Treponema pallidum* (21)
- *Mycobacteria tuberculosis* (17, 58, 139)
- *Mycobacteria leprae* (119)
- *Borrelia burgdorferi* (128)
- *Legionella pneumophila* (12)
- *Escherichia coli* (116)
- *Clostridium difficile* (56, 69)
- *Listeria monocytogenes* (14)
- *Entamoeba histolytica* (98)
- *Clamydia psittaciu* (123)

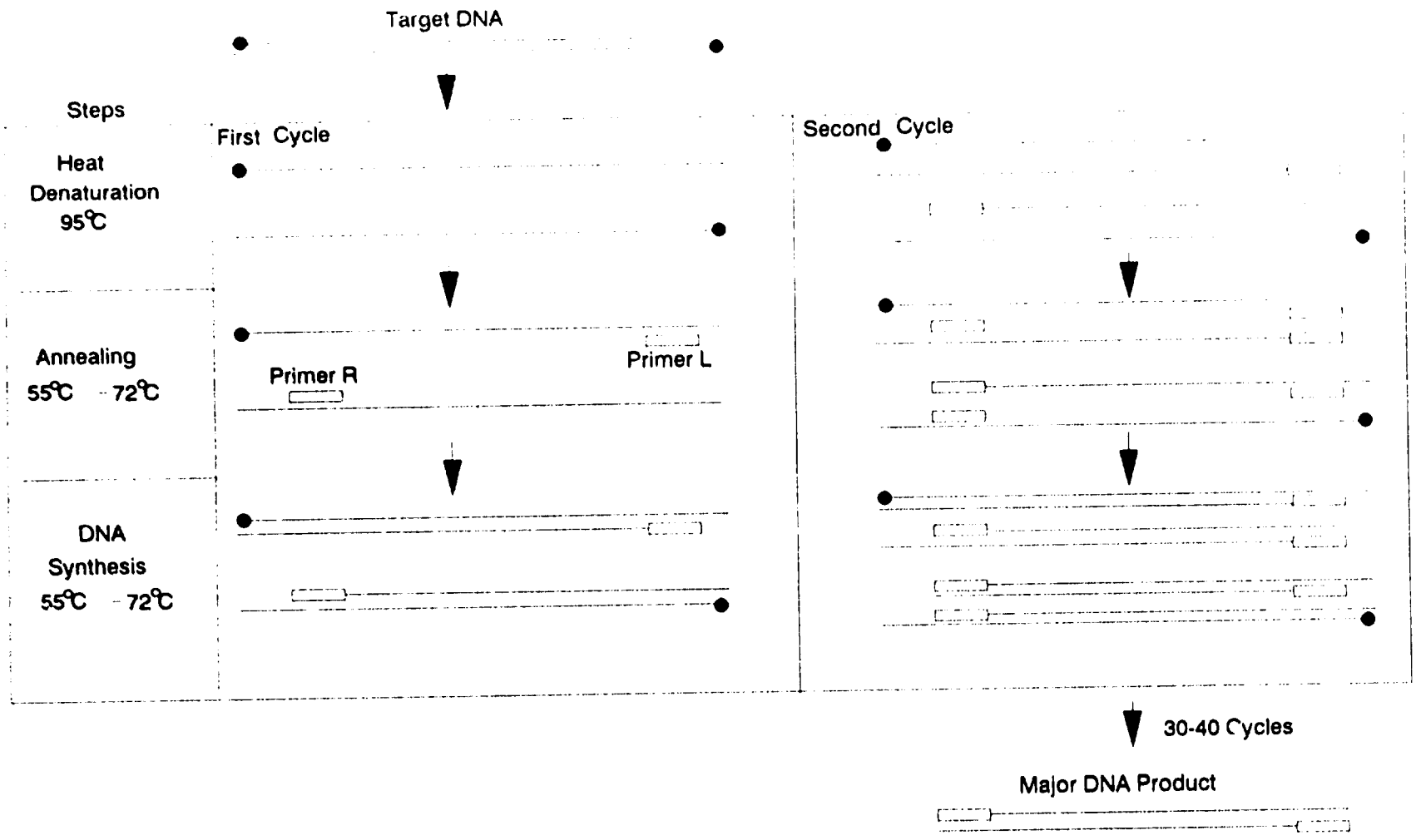
Target DNA
Steps
First Cycle
Second Cycle
Heat
Denaturation
95℃
Annealing
55℃ - 72℃
Primer R
Primer L
DNA
Synthesis
55℃ - 72℃
30-40 Cycles
Major DNA Product

FIGURE 1

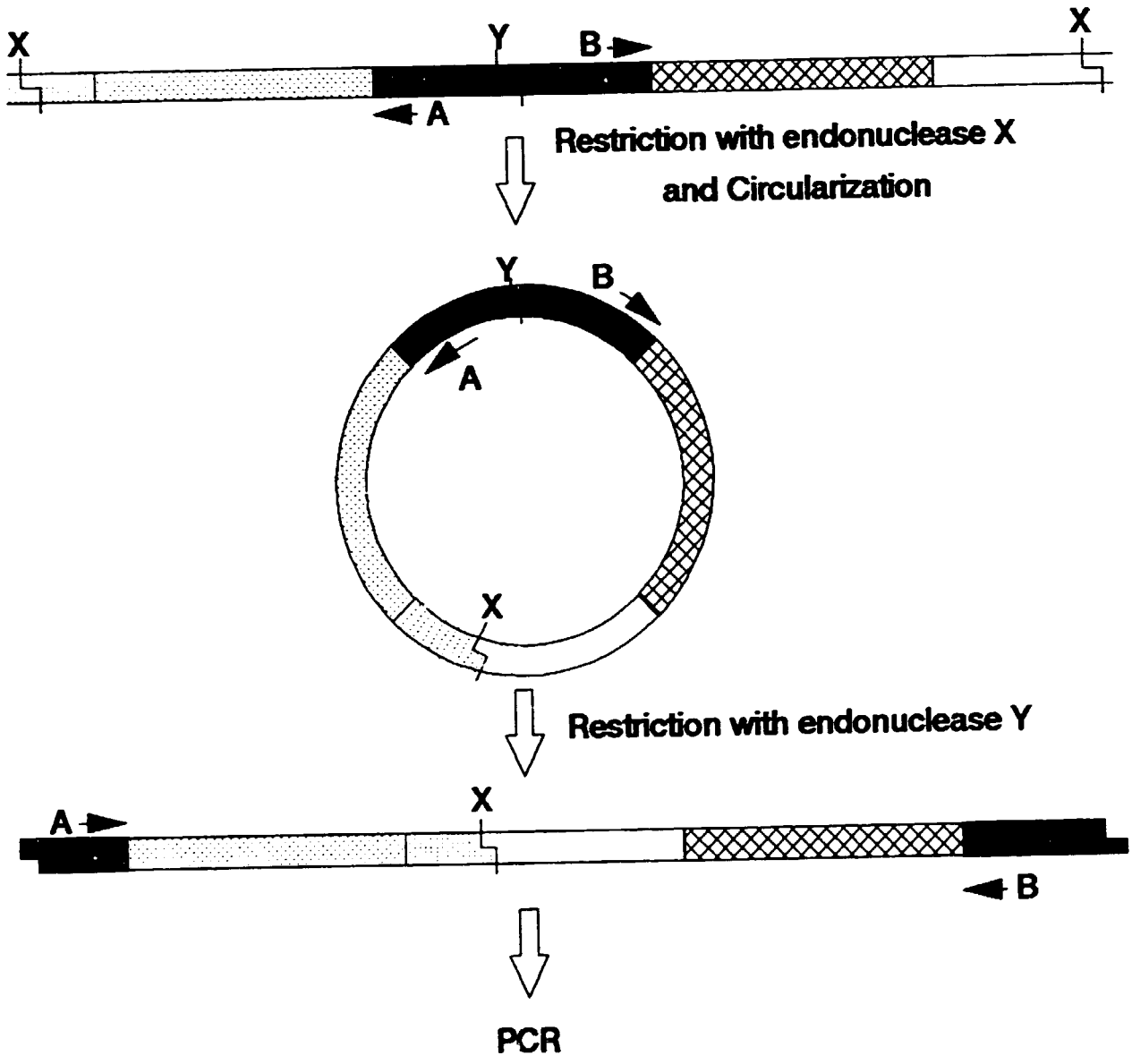

FIGURE 2

Amplification strategy to obtain sequences flanking a known DNA sequence. PCR primers A and B, shown in a 5' to 3' orientation, are located in a DNA segment with known sequence. Restriction endonuclease X and Y recognition sites are located outside and inside, respectively, the known DNA sequence. Digestion of the original DNA fragment with X followed by ligation generates a double stranded DNA circle. Linearization of the circular DNA puts the flanking unknown sequence between the two PCR primers.

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