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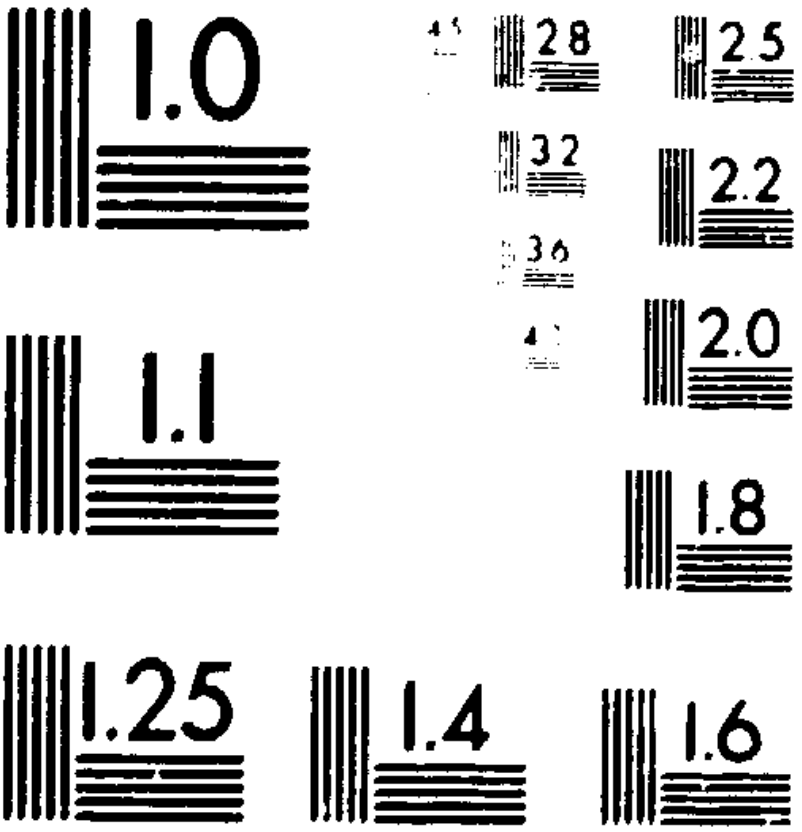

Genetic Engineering and Biotechnology Monitor

Issue No. 33 January 1991

N.B. This issue carries a reprint of an article on vaccines for the Third World by Dr. Barry R. Bloom, Chairman of the Department of Microbiology and Immunology at the Albert Einstein College of Medicine of Yeshiva University, New York, USA. The article was first published in Nature, and is reprinted with the kind permission of the author and publisher.

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A. POLICY, NEWS AND OTHER EVENTS

UNIDO News

ICGEB nears autonomy

The International Centre for Genetic Engineering & Biotechnology, with twin facilities at Trieste, Italy, and New Delhi, India, is on the threshold of autonomy, according to its preparatory committee. Only four more countries out of 24 need to ratify the Centre's statutes for them to come into force. The United Nations Industrial Development Organization has fostered ICGEB from its inception as a way for biotechnology research to serve the needs of third world nations. Currently, 41 nations, ranging from Afghanistan to Zaire, are members of ICGEB. With the four additional ratifications expected, UNIDO would be in a position to hand over responsibility for the running of ICGEB to the member States themselves. (Reprinted with permission from Chemical and Engineering News, 30 July 1990, p. 18. Copyright (1990) American Chemical Society)

Global guide on safety procedures

Four United Nations agencies are currently compiling a global training manual on safety procedures for organizations using genetic engineering techniques, to be issued by the UN International Centre for Genetic Engineering (ICGEB).

The manual will be targeted particularly at science-based industries in developing countries; one of its main aims will be to boost public confidence in the safety aspects of biotechnology.

The training manual will be compiled by a scientific working party on biotechnology safety, with members from the United Nations Industrial Development Organization (UNIDO), the United Nations Environment Programme (UNEP), the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO).

The working party has two objectives: to help developing countries establish programmes on the environmental and industrial aspects of biotechnology; and to inform administrators and policy makers of biotechnology safety guidelines. (Source: European Chemical News, 24 September 1990)

List of ICGEB meetings and courses in 1991

Practical course on molecular biology and diagnosis of human papilloma virus	1-23 February Havana, Cuba	P. Amati, Rome
Practical course on RFLP's in plant breeding	4-22 February New Delhi, India	J. Bennett, ICGEB
Practical course on protein and peptide purification, microsequencing and biotechnological applications	11-27 March, Buenos Aires Argentina	J. Santome/O. Cascone Buenos Aires
Theoretical course on bacterial genetics	18-22 March, Trieste, Italy	T.J. Silhavy, Princeton
Theoretical course on human genetics	21-27 April, Trieste, Italy	G. Romeo, Genova
Theoretical course on yeast molecular genetics	8-12 May, Trieste, Italy	G. Tocchini-Valentine, Rome
Practical course on yeast molecular genetics	13-18 May, Trieste, Italy	C.V. Bruschi, ICGEB
International symposium on pseudomonas biology and biotechnology (jointly with SISSA)	16-20 June, Trieste Italy	E. Galli, Milan C.V. Bruschi, ICGEB
Theoretical course on genetically manipulated organisms: Safety in the laboratory and the environment (jointly with UNEP)	1-3 July, Trieste Italy	T.G.B. Howe, Bristol
Conference on recent developments in research and release	3-5 July, Trieste Italy	J. Beringer, Bristol G. Tzotzos, ICGEB
Practical course on computer applications in molecular biology	8-20 July, Trieste Italy	D. Brutlag, Stanford
Practical course on plant transformation	4-22 September New Delhi, India	K.K. Tewari, ICGEB
Practical course on techniques in genome research	22-27 September Trieste, Italy	L.L. Cavalli-Sforza, Stanford F.E. Baralle, ICGEB

List of ICGEB meetings and courses in 1991 (cont'd)

Practical course on nucleic acid synthesis and gene assembly	4-22 November New Delhi, India	Q. Wang, ICGEB
Workshop on rDNA technology in the production of β-Lactam antibiotics	17-20 November Belgrade, Yugoslavia	V. Glisin, Belgrade
Theoretical course on marine microbiology and biochemistry (jointly with UNEP)	16-20 December Trieste, Italy	M.L. Sinnott, Chicago S. Paoletti, Trieste

Information: c/o Ms. Diana Viti, ICGEB Padriciano 99, I-34012 Trieste, Italy. Telephone: +39 40 3757333. Telefax: +39 40 226555, Telex: 460396 ICGEBT I.

The following collaborative research programme 1990 grants were awarded by the ICGEB

Country	Title	Project leader	Duration
Argentina	Ionic channels in plant cells: Molecular basis for plant improvement in semi-arid regions	F.J. Barrantes	3 years
Brazil	Oncogenes and anti-oncogenes in cell proliferation control	Hari C.S. Armelin	3 years
Bulgaria	Molecular basis of cystic fibrosis in Bulgaria	Luborodna Kalaydjieva	3 years
Chile	Studies of the stress response in biomining microorganisms. Possible implications in the improvement of the bioleaching process	Carlos A. Jerez	3 years
Chile	Saccarification of straw: use of enzymes from native fungi	Jaime Eyzaguirre	3 years
China	A novel, efficient and powerful method for site-specific mutagenesis	Qi Song Wang	3 years
China	Studies on structural mechanism of prolonged-acting and highly potent human insulin	Da Cheng Wang	3 years
Cuba	Transformation of sweet potato (Ipomonea Batata L) for increasing its nutritional value as food and animal feed	Sergio Perez Talavera	3 years
Greece	Photosynthetic water cleavage and inhibitory effect of herbicides	Demetrios F. Ghanotakis	3 years
Greece	Structural and functional analysis of human glutamate dehydrogenase	Nicholas Moschonas	3 years
Hungary	Characterization of DNA binding proteins involved in the regulated expression of a wheat chlorophyll a/b binding protein	Ferenc Nagy	3 years
Hungary	Structural studies on sequence specific DNA-protein interactions	Sandor Pongor	3 years

The following collaborative research programme 1990 grants were awarded by the ICGEB (cont'd)

Nigeria	The biology of bananas, plantains and of sigatoka in in the breeding for resistance to the sigatoka leaf spots	Tunde Fatunla	3 years
Nigeria	Screening of the antimutagenic and genotoxic activities of extracts of several edible vegetables plants and mushrooms commonly consumed in Nigeria	Emmanuel E. Obaseikhebor	2 years
Venezuela	A pilot project of the application of nucleic acid probes to malaria diagnosis in Venezuela	Hilda A. Perez	3 years
Yugoslavia	Molecular Diagnostics of Genetic and Infectious Diseases	Ana Savic	3 years
Yugoslavia	Sequencing by hybridization: Method development on gamma vectors	Radomir Crkvenjakov	3 years
Yugoslavia	Genetic and protein engineering of penicillin acylase	Vladimir Glisin	3 years

United Nations and other organizations' news

Fellowships in biotechnology

The Programme in Biotechnology for Latin America and the Caribbean (BIOLAC) of the United Nations University offers training scholarships for periods of 3 to 12 months in the following areas: genetic engineering of plants, micro-organisms for industrial use, and diagnosis and vaccines for human and animal health. The candidates should have, at least, a Bachelor degree or equivalent and some research experience. BIOLAC also offers partial funding up to US$5,000 for courses in these subjects. The programme also includes fellowships of 2 to 3 months duration, at several laboratories located in Argentina, Cuba, Mexico, and Venezuela.

For information, contact Dr. Camilio Daza Ramirez, Co-ordinator, Programme in Biotechnology for Latin America and the Caribbean, the United Nations University, Avenida Principal, Urbanización Cumbres de Curumo, Caracas 1080, Venezuela. (Source: Boletin de Biotecnologia, Vol. 7, No. 1, July 1990)

General

Human gene therapy tests

Medical researchers expect to carry out the first authorized tests of gene therapy on human patients later this year.

Dr. French Anderson of the US National Institutes of Health, plans to correct a rare inherited defect in the immune system, known as ADA deficiency, and Dr. Steven Rosenberg, another NIH researcher, hopes to use gene therapy to treat melanoma, the most dangerous form of skin cancer.

Medical researchers in the US have been keen to carry out human gene therapy since performing successful animal tests in the mid 1980s, but they have had to overcome a formidable array of regulatory obstacles.

The first trials of human gene therapy involve blood cells because these can most easily be removed from the body, have new genes added and then be put back into the patient. The NIH scientists have adapted a retrovirus to carry the genes into human blood cells. However, the procedure with retroviruses is cumbersome and although the viruses are genetically disabled to make them harmless, there are still residual fears about their safety. In the long run, gene therapists will insert new genes directly into cells without using retroviruses. An experimental "gene gun" which fires tiny capsules of DNA (genetic material) into cells was recently tested on mice at Duke University in North Carolina; Du Pont, the US chemical company, has commercial rights to exploit the gene gun.

Anderson hopes to cure ADA deficiency, which leaves children defenceless against infection, by inserting the ADA gene that is missing from their cells. ADA is an enzyme that is essential for the development of the immune system.

Anderson's procedure is aimed at correcting an inherited genetic defect. Gene therapy could in theory be developed to treat many of the thousands of known genetic diseases. One of the most far-reaching developments would be a treatment for the millions of people who have inherited an increased susceptibility to heart disease. US researchers have already taken a step in this direction by using gene therapy to lower cholesterol levels in the blood of laboratory rabbits.

The other NIH gene therapy test - the melanoma procedure - is not intended to correct an inherited defect; it is in effect a radically new drug delivery system. Rosenberg plans to take tumour-infiltrating lymphocytes - white blood cells that seek out and attack cancer cells - and add a gene that will make them produce a protein called tumour necrosis factor (TNF). The idea is that the TNF will give the lymphocytes enough extra punch to kill all the cancer cells.

If Rosenberg's procedure works, it will open the way to treating other forms of cancer, and indeed other diseases, by turning the body's own cells into miniature drug factories. This approach may be more effective than the current practice of the biotechnology-based pharmaceutical industry, which is to make proteins in cell cultures, extract and purify them, and sell them to doctors to inject into patients. (Extracted from Financial Times, 9 August 1990)

Dublin hosts world forum on biotechnology

The biotechnology industry in Europe could face severe problems in the near future unless the current negative climate changes, a world forum of the industry agreed recently.

The forum, which included some 100 senior industrialists, bioethicists, and researchers from around the world, met in Dublin Castle under the auspices of BioResearch Ireland to discuss the problems currently facing the industry, paramount among which are political climate and public attitudes.

The forum agreed that if the current climate does not change, Europe will also lose scientists and investments to countries like the USA and Japan. Currently, the rate of application of biotechnology in Europe was already low, with only 19 per cent of world patents originating there.

As to public awareness, the forum agreed that "the message just is not getting through. The public need to be informed."

Actions agreed by the Dublin meeting were the establishment of a Europe-wide forum to include the directors of all the national biotechnology programmes. Its first meeting will discuss the actions to be taken at national and international levels to improve public awareness and understanding of biotechnology.

The Dublin forum also suggested that the feasibility of a large-scale demonstration project should be investigated. This would probably be based in a developing country and among the potential examples cited was developing new strains of crops to tolerate arid conditions.

Finally, the Dublin forum also agreed the need to establish an independent, authoritative body to oversee aspects of the industry. A suggested model was the USA's Office of Technology Assessment (OTA), although this is unlikely to be appropriate for the European context. (Source: Technology Ireland, July/August 1990)

The cost of AIDS

When AIDS was incurable, it mattered little how much different treatments cost. Now cost matters, because of the onset of the disease in those who have caught the virus can be greatly delayed - perhaps prevented - by drugs. Treating somebody who already has the disease costs $20,000-$60,000 a year, depending on the available outpatient services. Pre-AIDS treatment now costs as little as $5,500 a year, down from about $9,600 last September. Yet many of those who need pre-AIDS treatment cannot afford it. This, for the first time, gives government an economic incentive to step in and help them - to save itself the later, larger bill for treating the disease.

In February the US Federal Drug Administration approved the use of zidovudine (which contains AZT) in people with fewer than 500 CD4 white blood cells per cubic millimetre of blood, rather than the previous 250. Half of all infected people are believed to have counts below 500.

Two things have combined to make drug treatment for them much cheaper. The recommended dose of zidovudine has been reduced from 1,200 to 500 milligrams a day; Burroughs Wellcome has lowered its price by 20 per cent. A year's supply of the drug now costs $2,750.

The treatment taken by people whose CD4 count drops below 200 to prevent pneumonia has also become cheaper. Such people receive either the aerosolised pentamidine for $2,500 a year or the trimethoprim-sulfamethoxazole (one familiar trade name is Septra) for $250. Many doctors are shifting to the older drug, since new findings suggest that is not only as good, but better. Still, about 20 per cent of patients are allergic to the old drug, and must stick with aerosolised pentamidine.

Only half of the people believed to have HIV have been tested and told. After the announcement in August 1989 that a preventive medicine existed, doctors expected an increase in people seeking HIV tests. The rush never materialized, or at least not at the publicly financed centres where the vast majority of tests are believed to be given.

Only half of those who know have health insurance to pay for it. Mr. Henry Greely, of Stanford Law School, estimates that roughly 50 per cent of pre-AIDS people have private, employment-related health insurance. It should cover 80 per cent of their costs; they will have to pay for the rest.

The other pre-AIDS people can only stand and wait.

If no one pays for a patient's treatment now, US federal and state governments will pay for it later, when he or she comes to hospital with an acute illness. That is expensive. These costs may fall, but so may the costs of prevention, if new drugs, such as dideoxyinosine and inosine pranobex, prove safe and effective. (Source: The Economist, 18 August 1990)

Proposal for world-wide DNA profiling network

ICI's Cellmark Diagnostics unit is planning to set up a world-wide network of DNA fingerprinting laboratories in an attempt to co-ordinate and evaluate the accuracy of different laboratories using different methods.

Questionnaires are being sent out to 50 laboratories to ask them how they feel about setting up such a network, where results of tests would be co-ordinated and assessed by Cellmark for a fee. Cellmark has been considering the scheme for some time, but was waiting for confirmation of the standard of their laboratory procedures before offering themselves as a quality assurance service. That confirmation came when Cellmark was awarded the British Quality Standard (BS 5750), the European Standard (EN 29002) and the International Standard (ISO 9002) for its fingerprinting procedures.

Cellmark's plans to set up the network have been accelerated by controversy over the reliability

of DNA fingerprinting techniques used as forensic evidence in criminal court cases in the US last year. In two cases DNA data were not admitted as evidence. In the first case, the match between two samples was not considered to be good enough, and in the second the testing laboratory's procedures were said not to follow set guidelines.

The scheme will be co-ordinated by Cellmark for a fee yet to be determined, and will entail each participating laboratory sending in a DNA sample, together with its own fingerprinting results. The sample will then be sent for testing to every other laboratory in the network, and the results will be pooled by Cellmark.

Each laboratory would be asked to send in samples for assessment every two or three months. Only the laboratory concerned would be told if its results were found to be inaccurate. (Source: European Chemical News, 20 August 1990)

Senior Advisory Group on Biotechnology established in Europe

The Senior Advisory Group on Biotechnology is an industrial forum, comprising board members of the major pharmaceutical companies, Ferruzi (Italy), Hoechst (Germany), ICI (UK), Monsanto Europe (Belgium), Rhône Poulenc (France), Sandoz (Switzerland), and Unilever (UK and The Netherlands). The group was established by the European Chemical Industry Federation to "promote a supportive climate for biotechnology in Europe". The Group has now issued a position paper entitled "Community Policy for Biotechnology: Priorities and Actions" that specifically addresses the so-called "fourth hurdle" that acts as a barrier to marketing of biotechnology products. It appears that European authorities are requiring not only demonstration of product quality, safety, and efficacy, but proof of socio-economic needs. This is a criterion required of other high-technology products such as pharmaceuticals produced by traditional chemical methods. The group recommends that regulators should judge products on the basis of the nature of the product, not the nature of the process by which it is produced. (Source: ABA Bulletin, Vol. 5, No. 4, August 1990)

Latin American Federation of Biotechnology Enterprise Associations: FELAEB

The first Latin American Federation of Biotechnology Enterprise Associations (FELAEB) was recently formed in Buenns Aires, Argentina. Business representatives from Argentina, Brazil, Chile, Costa Rica, Mexico, and Uruguay participated. Dr. Antonio Paez de Carvalho of Brazil was named president, and the Executive Secretariat will operate from Buenos Aires.

The objective behind founding a federation of national associations is to bring Latin American biotechnology enterprises closer together, to increase the exchange of experiences, and to organize a forum and a biennial or triennial Latin American biotechnology fair. (Source: Boletin de Biotecnologia, Vol. 7, No. 1, July 1990)

Agrobiotechnology in Central America

Dr. Walter Jaffe, specialist in Generation and Transfer of Technology for IICA, made a study to evaluate the capacities in agrobiotechnology in Central America. The work contains information on the organizations involved in the subject, manpower, distribution of physical resources and funds, types of technology used, groups of organisms studied, and businesses that use agrobiotechnology.

Twenty-six organizations related to this field were identified in the five Central American countries, and three more in Panama. Costa Rica and Guatemala showed the greatest development. Almost all the laboratories apply plant culture techniques in vitro; molecular biology and recombinant DNA techniques are in use only in Costa Rica. (Source: Boletin de Biotecnologia, Vol. 7, No. 1, July 1990)

Current situation of plant biotechnology laboratories in Latin America and the Caribbean

The regional office of the FAO for Latin America and the Caribbean (FAO/RLAC), organized a regional survey to evaluate the degree of development in the field of plant biotechnology. Visits were made to 153 laboratories belonging to both public and private institutions in fifteen selected countries.

The report contains information on manpower, the principal activities of each lab, plant species having priority, biotechniques in use, publications, infrastructure, and sources of financing. The report is available as a catalogue dated 1990 and may be requested from Dr. Juan Izquierdo, Oficina Regional Producción Vegetal, FAO, Apartado 10095, Santiago, Chile. (Source: Boletin de Biotecnologia, Vol. 7, No. 1, July 1990)

HUGO gains momentum

The Human Genome Organization (HUGO) has begun to make headway two years after its inception with the announcement of its first significant grants. The Howard Hughes Medical Institute recently announced a grant of US$1 million to be spread over four years, and Burroughs Wellcome is also expected to announce a grant soon.

At the same time it was announced in London that Sir Walter Bodmer, Research Director of Britain's Imperial Cancer Research Fund, will become HUGO's President, taking over from founding president Dr. Victor McKusick.

HUGO has 250 member organizations in 23 countries working together to elucidate the complete human genome in an envisaged 15 year time frame. The total cost of the project is estimated at $2,000-$4,000 million over the 15 years.

HUGO has invited any scientists or organizations interested in co-operating on the project to liaise through one of its three regional offices. HUGO is co-ordinating the sequencing research and facilitating the exchange of information, and has some funding at its disposal.

The HUGO offices are:

Hugo America, Ms. Diane Hinton, Howard Hughes Medical Institute, 6701 Rockledge Drive, Bethesda, MD 20817, USA. Telephone: (301) 571 0282, Fax: (301) 571 0573. HUGO Europe, Dr. Bronwyn Loder, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK. Telephone: (71) 269 3610, Fax: (71) 831 6265. HUGO Asia,

Dr. Kenichi Matsubara, Institute for Molecular & Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan. Telephone: (81) 6 877 5044, Fax: (81) 6 875 2468. (Source: Australian Journal of Biotechnology, Vol. 4, No.3)

European genome project re-emerges

Following delays of over a year, the European Human Genome Analysis Project has been revitalized with the backing of the European Council of Ministers. The European Commission has promised backing of $15 million over two years, with the majority of funds going towards actual mapping and computational analysis. Bronwyn Loder of the Imperial Cancer Research Fund (London) will co-ordinate the project.

In the near term, collaborating laboratories will produce a genetic map at the 1-5 centimorgan level. Specific project proposals will be solicited within a few months, when the Council's position has been approved by the European Parliament. (Source: Bio/Technology, Vol. 8, June 1990)

Human Genome Project - data base goes on-line

Geneticists in Oxford tested a new computer data base for the Human Genome Project. The data base was developed by a team led by Richard Lucier from Johns Hopkins University, Baltimore, Maryland.

The new data base will contain basic gene-linkage data, information on polymorphism, and practical details on genetic probes and the location of laboratory material. The genetic sequences themselves are deposited in the Genbank data base at the Los Alamos laboratory, but discussions to allow the exchange of data with Los Alamos are under way.

Similarly, the data base will be linked up to the existing On-line Mendelian Inheritance in Man data base, the leading repository for human genetic disease data (also at Johns Hopkins) and, it is hoped, the mouse genome mapping data base at the Jackson Laboratory in Florida.

Initially, copies will be held on mainframe computers at Johns Hopkins, and at a site in the United Kingdom - probably the Medical Research Council's Clinical Research Centre in North London. But there are plans to extend the service to the German Cancer Centre in Heidelberg and later to Japan and Sweden, so that geneticists in those countries will not have to depend on sometimes unreliable international electronic communications to access the genome data. (Source: Nature, Vol. 347, 6 September 1990)

EFB elects Latvia's biotechnology complex to full membership

In what may be the first quasi-diplomatic recognition of a break-away Soviet republic, the European Federation of Biotechnology has elected Latvia's Republican Interbranch Scientific Complex, Latvijas Biotehnologija, to full membership. The Federation's General Assembly took this action on the eve of the 5th triennial European Congress on Biotechnology, in Copenhagen Denmark.

The assembly also elected to membership the German Association of Physical and Mathematical Biology, and the Flemish-language branch of Belgium's Biotechnology Association. (Extracted from McGraw-Hills Biotechnology Newswatch, 16 July 1990)

Biotol: Open learning offers flexible bio-industrial training

In an attempt to address the developing skills shortage in biotechnology, the Thames Polytechnic (UK) and Open University (The Netherlands) have produced a biotechnology training scheme - using open learning materials. Biotol, which is part of the European Community Education and Technology Training (COMETT) offers training at graduate level through a Europe-wide credit accumulation and transfer scheme.

Biotol covers a wide range of subjects, from process biotechnology and genome management to their practical applications in agriculture, the environment and medicine. There are five sequential study themes: genetics, biochemistry, cell biology, physiology and technology.

Target groups include: technicians needing further education; recent graduates wishing to extend their knowledge base and potential; mature staff faced with changing work or a new career; managers unfamiliar with the new technology; and those returning to work after a career break.

Details from: Sarah Caffyn, Butterworth-Heinemann Ltd., P.O. Box 63, Westbury House, Bury Street, Guildford, Surrey, GU2 5BH. Tel: (0483) 300966. Fax: (0483) 301563. (Source: Biotechnology Bulletin, Vol. 9, No. 6, July 1990)

ASTM subcommittee seeks participants for standards activities in identification of viruses, cells, plasmids, fungi and bacteria

The ASTM Subcommittee E48.02 on Characterization and Identification of Biological Systems seeks participants to aid in developing standards for the identification and characterization of viruses, cells, plasmids, fungi, and bacteria, as well as for the preservation of biological samples.

Subcommittee E48.02 of Standards-writing Committee E-48 on Biotechnology has developed standard guides for the characterization and identification of the bacterial virus lambda and the animal virus herpes simplex virus. A standard guide for detection of the human immunodeficiency virus by polymerase chain reaction is being planned.

For more information, contact Larry E. Bockstahler, Food and Drug Administration, Rockville, MD 20857, 301/443-7287; or John Vowell, ASTM, 1916 Race St., Philadelphia, PA 19104, 215/299-5496.

Committee E-48 is one of 134 ASTM technical standards writing committees. Organized in 1898, ASTM (American Society for Testing and Materials) is one of the largest voluntary standards development systems in the world. (Source: News Release, 23 July 1990)

Biomass Users Network

The Biomass Users Network (BUN) is an international not-for-profit membership organization created by and for developing countries. BUN helps member countries to identify opportunities for improving rural economies while protecting natural resources and facilitates information dissemination, scientific and technical co-operation, and funding for demonstration projects in biomass production and utilization.

The concept of BUN emerged from a conference on "Bioenergy Approaches to National Development" held in Manila, Philippines, in March 1983 where representatives from 17 developing countries expressed the need for an organization that focused on South-South co-operation to address rural economic and natural resource degradation problems. In late 1985, BUN was formally inaugurated in Bangkok, Thailand.

Biomass is "... the basis for survival, the source of most income, and protector of the environment. Poverty ..." is simply a shortage of biomass. (Anil Agarwal and Sunita Narain in Towards Green Villages). Member-Countries who created BUN recognized that:

- Developing countries have a comparative advantage in biomass production because of their climatic conditions and relatively cheap labour in rural areas.

- Industrialized as well as developing countries have established economically viable value-added industries that produce and process biomass into pulp and paper, for example. The range of products that can be derived from value-added processing of biomass is constantly increasing as a result of scientific and technological advancements.

- Ecologically sound biomass systems that use cost-effective technologies in value-added processing facilitate sustainable use of renewable natural resources (soil, water, and plants) to generate income.

There is growing awareness that:

- Subsistence and ecologically degrading agriculture, in addition to land-intensive livestock production systems, is a major factor in destruction of natural resources (soil, water, and biological resources, including forests) in developing countries. Approaches to sustainable rural economic development should assist farmers to achieve what is economically rewarding, with minimal self-sacrifice, while protecting the natural resource base.

- With growing populations and limited land resources in most developing countries, sustainable development approaches must minimize the number of persons who derive their livelihood from primary agricultural production. Biomass systems are one of the most promising approaches for achieving off-farm employment in rural areas through local value-added processing.

- Effectively expanding value-added opportunities in the biomass sector requires relatively secure and lucrative market niches.

BUN's project focus is on four key areas:

- Protecting, rehabilitating, and developing degraded and fragile lands;

- Revitalizing sugarcane producing and processing industries through diversification to promote environmental protection and economic development;

- Sustainable production and efficient use of biomass fuels;

- Promoting economically viable and environmentally sound utilization of agriculture and forestry residues.

(Source: BUN information circular)

B. COUNTRY NEWS

Australia

Sandoz forges Australian R&D link

Sandoz has agreed a research collaboration and licence agreement with AMRAD Corp. of Melbourne to develop the human therapeutic uses of Leukaemia Inhibiting Factor (LIF).

LIF is a naturally occurring protein, which was discovered at the Walter & Eliza Hall Institute of medical research (WEHI) in Melbourne. The drug is believed to have a number of potential therapeutic applications in the stimulation of blood-cell production (haemopoeisis). Foremost of these, is an ability to act as a potent stimulant of platelets, the blood elements responsible for clotting. So, the most likely first application of LIF is as a treatment of thrombocytopenias, or platelet deficiencies, a severe side-effect prevalent in cancer patients undergoing chemotherapy and radiotherapy.

Under the terms of the licence, AMRAD will receive research funding and benchmark payments tied to specific, scientific and development goals. (Source: European Chemical News, 1 October 1990)

Keeping "track" of take-all protection

A new "tracking" technique to monitor the soil bacteria that can reduce take-all disease of wheat, is being used for the first time in Australia in a series of field tests being conducted by the CSIRO Division of Soils. The technique, developed by specialists from Monsanto Company and tested extensively in the USA, represents a significant advance in research on the growth and survival of these bacteria in field soil, according to CSIRO soil scientist in charge of the Australian field tests, Dr. Maarten Ryder.

"The technique, recently approved by the Genetic Manipulation Advisory Committee (GMAC), involves attaching a genetic "tag" to the bacteria, so they can be distinguished easily from other similar soil bacteria" Dr. Ryder said. "In this way, we can follow the tagged bacteria in a field soil for the first time and accurately determine their effectiveness. We can find out how far they move in the soil, how quickly they multiply, their survival and the site on the plant root where they act to curb take-all."

The soil bacteria responsible for reducing take-all is a naturally-occurring biological control organism from the Pseudomonas group that was isolated by Dr. Ryder's team from wheat field soil. Trials conducted by CSIRO showed that the Pseudomonas organism controls disease in both glasshouse and field conditions. The two gene "tags" inserted into the bacteria are from a certified safe strain of E. coli, a bacteria found normally in humans and animals. In the USA, this genetic tagging method has been used successfully for the last three years, with field tests confirming its benefits for tracking the fate of the bacteria. The tests also showed that the numbers of

marked bacteria declined rather than multiplied by the second year of introduction and they had no adverse effect on the soil environment.

The first Australian field test with the marker gene will be conducted in a small isolated trial area and if this is as successful as anticipated, researchers expect the tracker technique to be used more extensively in other biocontrol experiments. At the moment, the biocontrol organism is grown in culture, which is then added to wheat seed in a special coating to make the bacteria stick onto its surface, according to Dr. Ryder. The coated wheat seed is sown using standard sowing strategies and rates. The field test with the tagged bacteria is expected to continue for the next 15 to 18 months. The survival of the bacteria will be monitored during summer and into the next winter season. (Source: ABA Bulletin, Vol. 5, No. 4, August 1990)

New ABA President

Mr. John Grace, Managing Director of AMRAD Corporation Limited in Melbourne - a newly emerging pharmaceutical biotechnology company - was recently appointed the new President of the Australian Biotechnology Association at its Annual General meeting in Surfers Paradise.

John Grace has a wealth of experience in industries concerned with food technology as well as those in the pharmaceutical and human health areas. He also worked for a time as a Business Manager with CSIRO's technology transfer arm, Sirotech Ltd.

The Association has not neglected its role in educating the general public about the science behind biotechnology and released late last year a series of pamphlets which are freely available and are being widely used by schools.

Professor Peter Gray of the Department of Biotechnology, University of New South Wales, has been appointed Vice-President. Professor Peter Gray has been at the forefront of biotechnology research in Australia during the last ten years and in recent years his research has centred around the commercialization of a new human growth hormone produced by new technological methods. Peter Gray was one of the founders of the Australian Biotechnology Association.

Dr. Martin Playne stepped down from the presidency at the same meeting after holding that position for the last four and a half years. Dr. Playne is a Research Scientist with CSIRO and was, with Peter Gray, one of the founders of the Association. He has taken a leading role in creating the Association and setting up its functions. In recognition of Dr. Playne's services to the Australian biotechnology industry during the last five years, the Association presented him with an Award of Merit for services to Australian biotechnology. This is the first such Award made by the Association. (Source: News Release, 16 October 1990)

Barbados

Biotechnology in Barbados

In Barbados and the other eastern Caribbean states, cassava and yam are intensively grown. The marketability of yam however was and still is threatened by a disease known as Internal Brown Spot (IBS). The disease was discovered in the 1960s. Research started at the University of the West Indies and was later continued by Caribbean Agricultural Research and Development Institute (CARDI).

During the 1980s, CARDI established a tissue culture laboratory in Barbados. The laboratory was initially developed to service a virus-tested yam multiplication project.

In 1984 CARDI started a Cassava for Livestock Feed Project with the objective to develop a commercial production system using high yielding varieties and mechanized practices. Between 1984 and 1988 more than 32 new varieties were imported from CIAT (CGIAR's Centro Internacional de Agricultura Tropical) and conserved in vitro at the tissue culture laboratory of CARDI in Barbados. After the serious Cassava Bacterial Blight disease reached Barbados in 1987, breeding work has been extended to improvement of disease resistance.

Recently CARDI extended its research programme to sweet potato, plantain, banana and Anthurium. Further information available from Dr. E. Chandler, Caribbean Agricultural Research and Development Institute (CARDI), P.O. Box 64, U.W.I. Campus, Cave Hill Campus, St. Michael, Barbados, W.I. (Source: Biotechnology and Development Monitor, No. 2, March 1990)

Cuba

Genetic engineering experiments on plants

The first plants that have been subject to genetic engineering will be introduced in Cuba as an experiment within the next eighteen months, in addition to other technical studies being done on how to improve the quality of crops.

During the 1980s Cuba began to produce a large number of plants for the textile industry, in a speedy biotechnological process whereby plants were cultivated in test tubes. Over the last few years plant factories have been set up to conclude work begun on how to improve banana, sugar cane and pineapple crops.

Genetic improvement gained from experiments in the textile industry has produced many improved and disease-resistant varieties of sugar cane on the island, and laboratory experiments currently being done will also provide ways of improving the disease resistance of the potato. (Source: National News Agency No. 146-147, August 1990)

Denmark

Ban on field release of gene-altered plants eased

Since the passage of the 1986 Environment and Gene Technology Act, in response to widespread popular opposition to biotechnology, public interest has largely died down and the Government is applying built-in loopholes to the law's across-the-board ban on genetically altered products.

The first permit granted under the new dispensation was for Maribo Seed Co., a multinational Danish firm, to field-test gene-altered sugarbeet, into which Monsanto Co., St. Louis, Mo., had inserted a gene that confers resistance to its broad-spectrum, non-selective glyphosate herbicide, Roundup®.

Maribo is field-testing the transgenic plant on small controlled plots in four countries of Europe - Denmark, France, Belgium and the UK. Planting took place last April and first-season results will be evaluated for such factors as germination, growth and sugar content.

Another gene that Monsanto implanted in the sugarbeet strain protects it against Rhizomania, a viral plant disease not found in Denmark, so no virus-challenge tests are planned.

Before authorizing the experiment, the Danish Parliament discussed the sugarbeet test and decided that it was "a good use" for genetic engineering.

Maribo provided Monsanto with the sugarbeet strains to be modified and is supervising this first field trial of the transgenic plant through its subsidiaries in all four European countries. (Extracted from McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

European Community

US/EC task force seeks mutual benefits

The first meeting of the joint European Community/US biotechnology research task force took place on 10-11 September in Washington, DC.

The initiative follows an agreement signed recently by EC Commission vice-president Filippo Maria Pandolfi and Dr. Alan Bromley, assistant to the US president for science and technology.

Its chief aims are to boost biotechnology research and improve information exchange and co-operation between the EC and the US in this sector.

The first meeting focused on two main areas where there is particular scope for collaboration: bio-data banks containing information related to genome analysis; and the development of in vitro tests for use in biological and pharmacological studies. A second meeting of the task force is scheduled for July next year; approximately two meetings per year are envisaged.

The international competitiveness of the European Community's biotechnology industry "has been jeopardized by the absence of a coherent policy towards this sector", according to a recent report. Published by the Club de Bruxelles, Bio-industries: What future in Europe? reviews the major sectors of the regulatory framework in the EC, together with existing action programmes.

The report concludes that the EC Commission must ensure that biotechnology innovations are given adequate protection, and that European companies are not tempted to move to locations outside the EC. "A rapid response must also be found to certain ethical questions and to problems related to protection of the environment." (Source: European Chemical News, 24 September 1990)

European R&D falls behind

A European biotechnology association has repeated its call on the European Commission to agree on a co-ordinated policy toward biotechnology, warning that the region's biotechnology R&D and investments are falling behind those of the US and Japan.

Using patents as an indicator for R&D activity, Senior Advisory Group for Biotechnology (SAGB) says European firms acquired only 19 per cent of recently documented biotechnology patents granted world-wide, against 41 per cent for US and 36 per cent for Japanese concerns.

Furthermore, the group's survey of commercial biotechnology investments in 1989 found that the majority occurred in the US - a significant portion of which was made by European concerns. These include both startup investments and major investments.

In a new report, the group stresses the need for a comprehensive commitment to biotechnology in Europe to prevent a further decline in its competitiveness. In the past, SAGB - part of the European chemical industry federation, CEFIC - has been critical of the lack of cohesion between various EC departments regarding a common biotechnology policy. As well as agreement on proposals being debated in Brussels, the group wants clarified patent protection legislation and a well-defined product registration system based on safety, efficacy and quality.

The global market for biotechnology-based products of Ecu 5 billion/year ($5.5 billion) could grow to Ecu 80 billion by the year 2000, according to the group. (Source: Chemical Week, 20 June 1990)

Euro-investments

As a response to a forthcoming research funding initiative under the European Economic Community (EEC) BRIDGE programme, BioResearch Ireland (Dublin, Northern Ireland) and HOM Consultancy BV (The Hague, the Netherlands) are promoting the establishment of the Animal Cell Technology Industry Platform (ACTIP). The BRIDGE programme should make significant funds available for animal cell culture research; ACTIP's initial work will be to represent industry interests in formulating projects and allocating funds.

ACTIP may also take a more overt lobbying role with respect to emerging EC rules for the patenting of animal materials and the marketing of transgenic animals. ACTIP's counterpart in plant biotechnology, the Green Industry Biotechnology Platform (GIBIP), has been active in advising European Parliamentary representatives on the implications of the directive on the deliberate release of genetically modified organisms. (Source: Bio/Technology, Vol. 8, May 1990)

Finland

Phenol-fighting bugs

The organism Rhodococcus chlorophenolicus was found to successfully bioremediate peaty soil and sandy loam contaminated with polychlorinated phenols (PCP's), in research by Mirja Salkinoja-Salonen at the University of Helsinki. The R. chlorophenolicus breaks the PCP's completely down to chlorine and carbon dioxide, eliminating the problem of toxic byproducts produced by other organisms. In the research, soil contamination had varied PCP concentrations between 30-600 mg/kg of dry soil. Peaty soil was inoculated at the bacteria concentration of 10^5-10^8 cells/gt of dry soil. For sandy loam, the inoculation concentration was 500 cells/gt. In the soils with light contamination, the mineralization rate 12-18 mg/kg of dry soil in four months. For heavy contamination, the rate was 130-250 mg/kg of dry soil. The soils did not contain R. chlorophenolicus prior to inoculation. The inoculants maintained viable numbers for more than a year. Future research will include improving the bacteria for survivability through genetic manipulation and selection. The bacterial enzymes

which remove the chlorine from the PCPs have been identified. After purification, the enzymes will be tested independently of the bacteria for their bioremedial activity. (Extracted from: Bio/Technology, Vol. 8, June 1990)

Japan

New methane generating bacterium

The Agency of Industrial Science and Technology's Fermentation Research Institute has discovered a new bacterium which generates methane while decomposing acetic acid. Scientists expect to use the bacterium in an efficient anaerobic water processing reactor system.

The bacterium itself is only two microns in length, but reproduces in a filiform linkage of up to 100 microns, making it easy to retain within the reactor.

Anaerobic water processing reactor systems run at high temperatures - 55-60° C. Under these conditions, the rate-limiting factor is bacterial growth rate. The new strain's growth rate is 2-3 times higher than conventional bacteria, resulting in a significant improvement in efficiency. (Source: Bio/Technology, Vol. 8, June 1990)

BIDEC changes to JBA

BIDEC (Bioindustry Development Center) has changed its English name to JBA (Japan Bioindustry Association). The foundation was established in 1987 as the first organization for academic/industrial collaborations in bioindustry. For the past three years, the foundation has played an important role in founding bioindustry in Japan and is now expected to promote international collaborations. (Source: Bio/Technology, Vol. 8, June 1990)

Malaysia

Biotechnology in Malaysia

Biotechnology research in Malaysia is generally carried out by public or semi-public research institutions. The research covers many aspects of biotechnology. There is an interest both at the research and the application front. Most of these activities are at the preliminary or basic stages.

A National Biotechnology Committee (NBC) was formed in 1985 by the Ministry of Science and Environment. Its policy is to promote and co-ordinate research and development efforts at specific centres of excellence selected from existing universities and Government research institutes. The concept of the centres was developed to combine research expertise and facilities that exist in various institutions in the interest of better fundamental research and applied research as a service to industry.

Rubber and palm oil

Private plantation and seed companies such as Sime Darby, United Plantations, and Guthries have already invested in tissue culture programmes for mass propagation of high yielding varieties of palm oil. The Palm Oil Research Institute of Malaysia (PORIM) and Unilever finance research for modifying the fatty acid composition of palm oil. The Rubber Research Institute (RRI) in Kuala Lumpur funds projects for upgrading natural rubber to higher value added products. One project involves the use of fungal enzymes to chemically alter natural rubber for producing adhesives. Through tissue culture two year old cloned plantlets are tested in test plots outside. Genetic engineering and alternative energy resource research are at the basic laboratory scale. Further details from Mr. Chee Kim Sam, Ministry of Science, Technology & Environment. 14 Fl/Wisma Sime Darby, Jaslan Rja Laut, Kuala Lumpur, Malaysia. Phone: (0)3-2938955. Fax: (0)3-2936006. (Source: Biotechnology and Development Monitor, No. 3, June 1990)

Rattan and mushrooms cloned

Two alternative crops, rattan and the Shiitake mushroom are expected to generate considerable export earnings for Malaysian farmers in the near future.

The current shortage of rattan, a climbing palm (now worth US $3,000 per ton) used for furniture manufacturing, has led to a massive harvesting of rattan from the forests. Consequently Malaysia is researching ways of increasing production and profits. However, as tropical forests are felled for their timber, wild rattan vines are felled too. There is even a shortage of planting material for the limited new acreage being planned. The Forest Research Institute in Malaysia has proposed to use tissue culture for the production of more plants.

There is a large gene-pool of rattan varieties in South-East Asia (480 out of the 600 known on earth), from which 104 are found in Malaysia. At present about 20 varieties are in commercial use. The world market for dried Shiitake mushrooms is estimated at 3 million kilogrammes. This mushroom variety is a temperate crop and found in many South-East Asian forests. In Singapore the Shiitake mushroom is grown under air-conditioning. The Malaysian farmers have been able to grow the mushroom on natural logs. Biotechnology research will be needed to perfect this method to make it more economical. (Source: Panoscope, January 1990)

The Netherlands

Tropical molecular biology
Interuniversity postgraduate programme

The Vrije Universiteit Brussel co-ordinates a postgraduate programme in molecular biology, oriented towards scientists from developing countries.

Since 1985 more than forty participants have graduated in this "Tropical Molecular Biology" programme, which is organized with the support of the Administration for Development Cooperation of Belgium.

Molecular biology pervades all aspects of classical biology, agronomy, veterinary science and medicine. It is the basic science supporting biotechnology. Training young scientists is imperative and the progress in these fields is such that numerous scientists need re-orientation or additional training.

Many countries cannot satisfy the great demand for such training programmes, due to a lack of facilities or trained personnel.

The Institute of Molecular Biology at the Vrije Universiteit Brussel (VUB) in Belgium felt that it could help fill the gap. In collaboration with other research centres it has organized a course in

molecular biology. This two-year course of Tropical Molecular Biology (TMB), offers postgraduate scientists in developing countries the unique opportunity to be personally involved in the rapidly expanding field of molecular biology and its applications to biotechnology.

The degree, a masters' degree in molecular biology, is awarded at the end of a successful completion of this two-year programme. The programme is oriented towards theory but also towards actual situations encountered in developing countries.

Formal course subjects and laboratory exercises include:

- Molecular biology
- Biochemistry
- Protein and nucleic acid chemistry
- Microbiology
- Animal and plant physiology
- Molecular plant genetics
- Somatic cell genetics
- Phytopathology
- Parasitology
- Immunology
- Virology
- Genetic engineering

The programme does not only involve the VUB but also other universities and centres in which research relative to developing countries is being undertaken.

The first year is entirely devoted to courses, practical exercises and visits to other research centres. Second year students perform an experimental project of at least 8 months. At the end of the academic year the students publicly defend their Master's thesis, as a partial fulfilment of the requirements for the Master's degree.

Ongoing research projects cover fundamental as well as applied topics in fields as diverse as plant and animal cell cultures, crop improvement (sorghum, sugar beet, maize), salinity tolerance in plants, somatic cell genetics, biocontrol of phytopathogens, tropical diseases (malaria, leishmaniasis, sleeping sickness), immunobiology (host-tumour and host-HIV interactions), vaccine development, hybridoma technology, practical applications of bioluminescence, antinutritive factors in foodstuffs, enzyme engineering etc.

Admission requirements:

- A four-year degree in science, medicine, veterinary science, agricultural engineering;
- A B.Sc. degree if the candidate has received additional training and/or has a broad research experience;
- A good knowledge of the English language is essential.

How and where to apply:

- Information may be obtained from the "Director-Tropical Molecular Biology programme", at the VUB (address given below);
- Admission to the course will be decided upon examining the completed application forms of the prospective candidate (with standardized recommendation letters and certified copies of diplomas).
- Tuition:
- For 1990-91 students from the majority of developing countries paid 11.500 BEF (presently approximately $US 330);
- Total annual expenses for studies in Belgium amount to approximately 300,000 BEF (including tuition, accommodation, food, additional clothing).

Available financial support in Belgium:

- One can apply for a scholarship at the Belgian Office for Development Co-operation (A.B.O.S./A.G.C.D.), through the local Belgian diplomatic representation. A limited number of scholarships (10) is granted yearly on a competitive basis;
- It is not possible to fund studies at the VUB with a research assistantship;
- A copy of the scholarship application should be forwarded to the director of the course.

General information:

The academic year begins in the first week of October; courses, practicals and examinations are scheduled until July.

For further information and application please write to:

DIRECTOR - TROPICAL MOLECULAR BIOLOGY programme
Vrije Universiteit Brussel
Paardenstraat 65
B-1640 St. Genesius-Rode
Belgium
Telephone: 00-32-2-358.34.17
Telefax: 00-32-2-359.03.90
Telex: 61051 VUBCO

Spain

Reduction and replacement of animal testing

A team of Spanish researchers has demonstrated "excellent potential for the reduction, refinement and replacement of animal experiments" according to the European Federation of Pharmaceutical Industries' Associations.

José Castell and Maria José Gómez-Lechón, of the hepatology unit of Hospital de la Fé, Valencia, have been investigating the use of cultured human tissue in studying the risks of new drugs. The team gained first prize in the EFPIA research awards, which this year were devoted exclusively to alternatives to animal testing. The jury said the Valencia work displayed a "consistent and original scientific approach".

"The use of human tissues obtained from biopsies and readily available cell lines represents major advances which are particularly commended" said the jury. Apart from avoiding the use of animals, the award-winning entry would give greater reliability in testing drugs, because of the use of human cells rather than animal cells, the jury added.

Results could also be obtained more quickly and, because smaller amounts of the drug compound are required to conduct the tests and no animals are needed, the costs would be reduced. The technique would therefore allow earlier testing for hepatotoxicity, the jury said. (Source: Chemistry and Industry, 2 July 1990)

The Philippines

Biotechnology in the Philippines

The Philippines is an example of a developing country where agricultural export markets are seriously threatened by current international developments in biotechnology. This applies to the export of coconut oil and sugar on which about 15 million smallholders depend. Coconut and sugar cane account for about 30 per cent of the cultivated area and for about 50 per cent of agricultural exports in the Philippines. Biotechnology is used to stimulate crop diversification, which could lower the vulnerability of traditional exports.

The most important biotechnology initiative has been the establishment of the National Institute of Biotechnology and Applied Microbiology (BIOTECH) within the University of the Philippines at Los Banos. General objectives have been the development of appropriate technology for bio-fertilizer (Rhizobium a.o.), food (tissue culture and fermentation), biopesticides and bio-fuels.

Bio-fertilizers are expected to offer the smallholders a cheaper alternative for chemical fertilizers on which agriculture in the Philippines heavily depends. Standard Rhizobium inoculum media are developed out of local ingredients like coconut water and sucrose, increasing the availability to farmers. Recipient crops are soybean, rice, corn and sugar cane.

Bio-pesticides (micro-organisms and viruses that attack insect pests and pathogens) are expected to replace chemical pesticides. Application is possible for controlling nematodes in banana, potatoes and other crops. Wide use is not achieved however due to high pricing of the private sector which controls the distribution.

Bio-fuels (ethanol and bio-gas from agricultural crops and residues) are considered to protect the country from increases in fossil fuel prices and environmental pollution. High capital costs however prevent large-scale application. It is expected that the direct use of sugar cane juice and sweet sorghum juice as direct feedstocks for ethanol fermentation will lower production costs in the long run.

Food biotechnology

Research in the field of food biotechnology is carried out by Natural Resources Research Institute (NSRI), the Industrial Technology Development Institute (IDTI) and the Philippine Council for Industry-Energy R&D. It is expected that through the installation of two government agencies within the Department of Science and Technology, the Philippinian Council of Advanced Science and Technology Research and Development (PCASTRD) and the Advanced Science and Technology Institute (ASTI), attention for food biotechnology will increase. Currently about 90 per cent of R&D spendings in the Philippines is financed by Government. Through financial incentives the Government hopes to increase private activities in R&D.

In the area of food biotechnology the private sector is represented by only a few companies. Private investment concentrates on cassava and fermentation industries. One private company, in co-operation with the University of the Philippines, concentrates on the commercialization of a fungus against root nematodes in several indigenous crops.

The University of the Philippines will set up a joint sugar cane biotechnology programme with the sugar producing industries. Further details available from Dr. L. M. Joson, National Institute of Science and Technology, P.O. Box 774, Manila, Philippines. (Source: Biotechnology and Development Monitor, No. 3, June 1990)

Trinidad and Tobago

Trinidad and Tobago

As a result of the substitution of sugar by high fructose corn syrup (HFCS), Trinidad and Tobago is one of the states in the Caribbean struck by serious drops in sugar exports. More than 10,000 sugar workers lost their jobs.

In order to lower production costs, biotechnological research in Trinidad and Tobago includes improvement of sugar cane yield and resistance. Attention is also paid to mussenda, aechimea and plantain. The work is concentrated in the University of the West Indies, St. Augustine. At the moment the staff consists of 12 researchers. Primary goal is greater food production. Four programmes are in progress, on yeast microbiology, mycorrhizal studies, bio-insecticides and plant tissue culture. Applications are on mass propagation and crop improvement, through selection and mutagenesis in vitro. Further information available from: Prof. E. J. Duncan, University of the West Indies, Department of Plant Science and Biochemistry, Faculty of Agriculture, St. Augustine, Trinidad and Tobago. (Source: Biotechnology and Development Monitor, No. 2, March 1990)

United Kingdom

Prescription for UK industry

Government should take a more pro-active role in the development of biotechnology in the UK, the Advisory Council on Science and Technology said in a report* released last June.

The report called on the UK Government to maintain a strong voice in EC policy talks, and said that the UK should resist recent EC moves to impose "discriminatory" restrictions on genetically engineered products.

The group believes that "skill shortages represent the most serious potential limiting factor" for UK biotechnology. ACOST wants the Department of Education and Science, the research councils and the Department of Trade and Industry to "urgently consider a co-ordinated approach with industry" to address the shortage.

The report also targets specific areas of research for intense development. Plant biotechnology, stem cell research and husbandry and animal health work, such as cattle embryology, are seen by the subcommittee to have great potential. (Extracted from: Chemistry & Industry, 2 July 1990)

SERC funds new interdisciplinary research centre on biochemical engineering

The Science and Engineering Research Council (SERC) has set up three further Interdisciplinary Research Centres (IRCs), covering cellular and molecular studies of simple nervous

* "Developments in biotechnology", £8.50, HMSO.

systems, biomedical materials and biochemical engineering. They are supported by 6-year grants totalling about £18 million. The third of these IRCs will be based at University College London (UCL) and bring together a consortium of collaborating departments within UCL, as well as groups at the Universities of Oxford and Kent. This particular centre represents a consolidation and conversion of existing SERC-supported groups at UCL. The principal location of the IRC will be in the Advanced Centre for Biochemical Engineering at UCL, to be completed in 1991.

The primary objectives of the Centre are: (1) the identification of general principles for processing of biological materials to accelerate the rate of definition and design of process operations; (2) the examination of the specific problems associated with the production and recovery of high value products, an area where the technological leverage to UK industry is felt to be particularly high; (3) to understand the scale-up of operations involving recombinant materials; and (4) to explore the implications of molecular biology for bioprocessing.

The total process compatability provided by existing facilities will be extended on completion of the advanced centre. This will house a unique large-scale category II biocontainment facility, the only university-based facility of its type in the world. This will allow a thorough exploration of the interaction between molecular biological and recombinant techniques with bioprocessing. The research will focus on biocatalysis, downstream processing and bioprocess design and operation. Details from: Science and Engineering Research Council, Polaris House, North Star Avenue, Swindon, Wiltshire SN2 1ET or on (0793) 411257. Fax: (0793) 411400. (Source: Biotechnology Bulletin, Vol. 9, No. 6, July 1990)

Interdisciplinary research centre in cell biology

The Medical Research Council (MRC), University College London (UCL) and King's College London (KCL) have jointly agreed to the setting up of an Interdisciplinary Research Centre (IRC) on Cell Biology. It will be based in a new purpose-designed accommodation at UCL and will also be part of the KCL development at Cornwall House. The IRC will establish an environment for cell biology ideal not only for research, but also for training postgraduate students and attracting outstanding post-doctoral scientists back to the UK. The emphasis will be on the application of molecular biological techniques to understanding cell function.

The director of the IRC will be Prof. Colin Hopkins, currently Rank Professor of Physiological Biochemistry at Imperial College, who will move to a Chair at UCL. Details from: Dr. Michael Kemp, Medical Research Council, 20 Park Crescent, London W1N 4AL or on 071-636 5422, ext. 6236. (Source: Biotechnology Bulletin, Vol. 9, No. 7, August 1990)

Release regulations

The UK Government's Department of the Environment (DoE) and the Health and Safety Executive (HSE) have jointly established a new body to advise on the release of genetically engineered organisms into the environment. The new Advisory Committee on Release to the Environment (ACRE) will replace existing committees in each department, a move that should eliminate the duplication of interests between agencies and help streamline regulatory procedures. (Source: Bio/Technology, Vol. 8, June 1990)

Release information

Bowing to pressure from environmentalist groups and members of the House of Lords, the UK Government is to amend the Environmental Protection Bill so as to ensure public access to information on proposed releases of genetically engineered organisms. The Government had originally argued that clauses on public access were not needed because the Environment Secretary already has the power to make this information public.

The new clauses propose public registers containing applications for consents to release, supporting information supplied with these applications, and any advice given to the Environment Secretary by the new independent Advisory Committee on Releases to the Environment (ACRE). Some information may be withheld if the Environment Secretary decides that this would undermine national security, provoke sabotage by extremist groups which would present an environmental hazard, or breach commercial confidentiality. But in commercially sensitive cases, the name and address of the applicant, a description of the organism nd the purpose of the release, and the results of any assessment of the environmental risks posed must still be included. (Source: Nature, Vol. 347, 11 October 1990)

UK virus field trial

Genetically engineered viruses that kill caterpillar pests are to be tested in an enclosed field in the UK for the first time in 1991. The baculoviruses, designed by researchers at the Institute of Virology and Environmental Microbiology, Oxford, UK, kill caterpillar pests that affect sugar beet and cabbage.

One of the two viruses to be tested contains a gene for a bacterial toxin. The residue from this virus persists to protect the plant from many different insect species. The other virus contains an insect enzyme that affects the pupation stage of caterpillars' growth. Both viruses are designed to self-destruct.

The trial was announced at the British Association meeting in Swansea, Wales, last August. (Source: European Chemical News, 3 September 1990)

United States of America

Eight states pass biotechnology laws in first half of 1990

Oklahoma became the eighth state this year to enact a biotechnology statute, when it passed the Oklahoma Agriculture Biotechnology Act on 15 May. This regulates field-release of genetically engineered organisms. Vermont and New Hampshire both defeated legislation that could have postponed approval of bovine somatotropin (BST), and another five bills to limit BST died in the legislatures of Virginia and Washington.

Oklahoma's newly enacted law, S.B. 228, establishes notification and licensing requirements for maintenance, transport, and environmental releases of "any organism altered or produced through genetic engineering", but it exempts from the Act "all persons who have filed Assurances of Compliance with federally established guidelines

with their Institutional Biosafety Committee, and/or applied for regulatory approval(s) from the appropriate federal agency." (Source: McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 13, 2 July 1990)

Biotechnology options weighed

The US Congress is forming a task force to look at the need for new legislation to regulate biotechnology and its impact on the environment. Meanwhile, the biotechnology science co-ordinating committee within the President's Office of Science and Technology Policy (OSTP) has published its proposed scheme outlining how key federal agencies will attempt to oversee biotechnology issues affecting the environment.

The immediate impetus behind the Congressional task force is twofold: the proposed amendments to statutes governing regulatory activities conducted by the US Department of Agriculture (USDA) affecting biotechnology; and the Omnibus Biotechnology Act, introduced in July by Representative Robert Roe, chair of the House of Representatives committee on science, space, and technology.

Among other goals, the Roe bill seeks to amend laws governing several federal regulatory agencies and would set uniform practices for permitting deliberate release experiments over the next seven years. It is also intended to make US regulatory practices closer to those in Western Europe.

Current plans call for the Congressional task force to review the Omnibus Biotechnology Act and other legislative options over the next six months. The task force hopes "to develop a consensus on what [biotechnology] legislation should look like".

The task force is expected to involve representatives from industry, universities and scientific societies, as well as federal agencies and Congress.

On a separate but related track, the administration's scope principles document was recently published in the Federal Register for public comment. The principles are being criticized for being too "vague and philosophical" because they throw important decision making back to individual federal regulatory agencies, but their acceptance could help break the logjam retarding the development and implemenetation of federal regulatory policies on biotechnology.

For instance, publication of the scope draft principles is expected to speed the publication of the proposed rules and guidelines from the Environmental Protection Agency (EPA) and the USDA. EPA officials say they will soon publish rules under the Federal Insecticide, Fungicide, and Rodenticide Act.

The scope document is supposed to help federal regulatory agencies to develop consistent policies "without unduly inhibiting" deliberate release experiments. The proposed new principles potentially extend regulatory jurisdiction to include genetically modified organisms "resulting from any process or technique".

According to the document, this choice of language is intended to avoid singling out any particular method, such as recombinant DNA techniques, and to correct any "misconception" that one method is "inherently of greater risk" than any other means of genetically modifying living organisms.

The document acknowledges that agency officials may sometimes need to consider the means by which organisms were genetically modified when evaluating their safety for release into the environment.

Oversight of planned introductions is to be "risk based", according to the scope document, and reserved for those experiments where "the risk posed by the introduction indicates that oversight is necessary". However, the draft principles "do not dictate precisely what information on risk must be considered". Instead, they "set forth general criteria for assisting agencies in developing possible categories ... for exclusion from oversight", leaving much of the implementation of the principles "within the discretion of individual agencies". Many traits of the organisms and of the environments into which they may be placed are mentioned as "relevant risk factors". Six possible categories of exclusion are described:

- Plants and animals that result from natural reproduction or by traditional breeding techniques;
- Micro-organisms modified solely through chemical or physical mutagenesis, by transduction, transformation, or conjugation, or by plasmid loss or spontaneous deletion;
- Vascular plants regenerated from tissue culture, including somaclonal variants, embryo rescue, protoplast fusion, or treatments that cause changes in chromosomal number;
- Organisms that have been modified by non-coding, non-expressed nucleotide sequences that cause no phenotypic or physiological changes;
- Organisms resulting from deletions, rearrangements and amplifications, within a single genome, including extrachromosomal elements; and
- Organisms with new phenotypic traits conferring no greater risk to the target environment than the parental strain, which is considered safe.

(Extracted from: Bio/Technology, Vol 8, August 1990 and Chemistry and Industry, 3 September 1990)

US grants will match Maryland firms with Thailand's bio-needs

Biotechnology companies in Maryland will get a chance to do business in Thailand, under a networking scheme announced by the University of Maryland's BioTechnology International (BTI) programme. The multi-agency plan involves both national governments, several state agencies and the private sector, as follows:

- The Royal Thai government's Science and Technology Development Board (STDB), which is funded by the US Department of State's Agency for International Development (USAID), has awarded BTI a $70,000 contract to survey opportunities for commercializing biotechnology in the south-east Asian country.
- Simultaneously, USAID has given $30,000 to the Maryland Office of International Trade (MOIT), to seek out companies in the state whose activities fit with Thailand's

needs. MCIT will match this federal grant with $30,000 worth of services.

(Source: McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 13, 2 July 1990)

RAC endorses two gene therapies; clinicals to start after FDA okay

One day after its Human Gene Therapy Subcommittee voted in favour of two gene therapy experiments in humans, the Recombinant-DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) on 31 July endorsed both clinical trials. The only obstacles remaining are final approvals by the acting director of NIH and the US Food and Drug Administration (FDA), which are expected in the next few weeks.

One experiment, which the RAC approved by a vote of 16 to 1, involves inserting the gene for adenosine deaminase (ADA) into blood cells taken from children suffering from severe combined immunodeficiency (SCID). The cells will be returned to the patients to produce the missing ADA enzyme in vivo. The RAC subcommittee had requested some additional information before it finally gave its endorsement.

In the other gene-therapy experiment, approved unanimously by the RAC, the gene for tumour necrosis factor (TNF) will be inserted in tumour-infiltrating lymphocytes (TIL cells) to destroy advanced melanoma tumours. This experiment, approved by the National Cancer Institute's biosafety review board on 14 May was delayed until RAC's July meeting, because of requests for additional information by the National Heart Lung and Blood Institute's review board. (Source: McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

Environmental biotechnology centre formed

The Centre for Interfacial Microbial Process Engineering is a new engineering research centre being established at Montana State University, Bozeman, with a grant from the US National Science Foundation. The Idaho National Engineering Laboratory, in Idaho Falls, is a partner in the centre, which, the university says, builds on work started seven years ago by its Institute for Biological and Chemical Process Analysis (IPA). Cross-disciplinary research, academics and technology exchange with industry, it says, will be the three foci of the new centre, as they are at IPA. The centre is developing state-of-the-art knowledge of interfacial microbial processes for application in biofouling and biocorrosion, bioremediation and eventually biohydrometallurgy. (Reprinted with permission from Chemical and Engineering News, 3 September 1990, p. 28. Copyright (1990) American Chemical Society.)

C. RESEARCH

Research on human genes

Immunex details hybrid biotech drug advance

At a meeting of the International Society for Experimental Haematology (ISEH), scientists from US biotechnology company Immunex reported the genetic engineering of a "fusion molecule". This is composed of two biotechnology drugs : granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL-3).

The Seattle-based company claims that in vitro studies show the fusion molecule to be about 10 times as active as a combination of IL-3 and GM-CSF, measured by its ability to promote bone-marrow cell growth. If proven safe and effective, Immunex fusion molecule, coded PIXY-321, could be the first of a second generation of colony stimulating factors (CSFs).

Previous animal studies have shown that sequential administration of the two cytokines (immune system modulators) could be synergistic in raising both white blood cell and platelet counts. However, the fusion molecule offers a potentially greater synergy than has been observed in studies combining GM-CSF and IL-3.

Cytokines activate target cells after attaching to a specific receptor on the cell's surface. Immunex postulates that the increased activity of the fusion molecule is due to its ability to bind a dual receptor, discovered by the company, in addition to receptors of IL-3 and GM-CSF.

The company has submitted a product licence application in the US for the use of GM-CSF in bone-marrow transplantation. In addition, phase I/II studies for reversing neutropenia (deficiencies of mature white blood cells) as a result of radiation or chemotherapy are under way. Phase I/II studies of IL-3 to determine its effect in neutropenia and platelet deficiencies are being conducted at centres in the US and Europe. (Source: European Chemical News, 10 September 1990)

Ys and wherefores

A group of British researchers announced the discovery of a stretch of DNA that is likely to be the "active ingredient" of the Y-chromosome in mammals: the gene that makes an embryo male. Despite its significance, the gene (named SRY) is remarkably tiny.

The discoverers, led by Dr. Peter Goodfellow of the Imperial Cancer Research Fund and Dr. Robin Lovell-Badge of the National Institute for Medical Research, both in London, think that SRY acts like a switch. Initially the sex organs of an embryo are the same in both males and females. After ten days gestation in mice, and seven weeks in people, they start to differ. It is believed that around this time SRY produces a small protein. The protein probably binds to one or more pieces of DNA that control the development of sex organs. If the SRY protein is there, nearby genes tell the organs to become testes, if it is not, they become ovaries. The rest of the sexual characteristics then depend on the hormones the sex organs produce.

To find which bit of the Y-chromosome is essential for maleness, researchers have studied people whose sex does not correspond to their chromosomes. Normally men have an X and a Y, and women have two Xs. However, some men have two Xs (one with a small amount of Y inserted into it); conversely, some women are XY (with something lacking from the Y). In theory, the piece of Y common to all XX men and lacked by all XY women would contain the relevant gene. The reality has proved to be more complicated. In 1987 a group at the Massachusetts Institute of Technology thought it had found the sex gene, but it had been misled by a tiny additional flaw in an XY woman's Y chromosome.

The SRY gene seems a much better candidate. It fits all the available data from XX men and XY women and it seems to make protein at just the right time

to influence the development of testes. SRY look-alikes exist in male mice, cattle, sheep, pigs and tigers. Parts of it resemble a counterpart in yeast.

The discovery poses more than ethical puzzles. Sometimes, parts of a pair of chromosomes can "cross over" from one to the other. SRY lies very close to the only part of the Y chromosome which ever crosses over with the X. That was fortunate for the researchers, because it produced the XX males and XY females they studied. Since these people are normally infertile, one might expect natural selection to favour putting the gene somewhere else on the chromosome. (Extracted from The Economist, 28 July 1990)

Invitron studies protein in multiple sclerosis model

In a study led by Halina Offner, associate professor at the Oregon Health Science University, and Dr. Arthur Vandenbark of the Veterans Affairs Medical Center, recombinant Immunomodulatory Lectin-1 (IML-1) prevented the clinical and histological signs of disease in an animal model for multiple sclerosis. IML-1 belongs to a family of carbohydrate-binding proteins termed lectins, which are involved in cell-to-cell communication. Lectins are critical components in the process of lymphocyte homing, the mechanism by which disease-fighting white blood cells are directed out of blood vessels and into tissues. IML-1 is thought to function in human pregnancy, where it may act to protect the foetus from "rejection" by the mother's immune system. Invitron has developed a process for manufacturing gram quantities of recombinant IML-1 in E. coli for use in studies being conducted by outside collaborators. In those studies, recombinant IML-1 has demonstrated immunosuppressive activity in animal models for several autoimmune disorders and transplantation rejection. Details from: Invitron Corp, 4649 Le Bourget Drive, St. Louis, Missouri 63134, USA. (Source: Biotechnology Bulletin, Vol. 9, No. 6, July 1990)

Cultivated brain cells

Improved treatment for central nervous-system impairments may result from an achievement at Johns Hopkins School of Medicine. The neurons comprising the adult central nervous system do not reproduce themselves. Once they are dead, replacement is impossible. Brain and spinal injuries are usually permanent. Scientists at Johns Hopkins have discovered a type of human brain cell that could be nourished and cultivated. A laboratory culture of the neurons was sustained and multiplied for almost two years. Cells from the laboratory grown culture could help scientists elsewhere understand what triggers diseases such as Alzheimer's, Huntington's and multiple sclerosis. Drug therapy development could be accelerated by studying the impact of various chemicals on neurons. Understanding how to introduce new genes into the cultured cells as a replacement for lost brain tissue may also be an outgrowth. (Extracted from Time, 14 May 1990)

Mabs used to remove cancer cells from bone marrow

Cancer cells can be removed from bone marrow with monoclonal antibodies and magnetic beads, according to Carole Heath of Dartmouth College. The technique allowed removal of 99.997 per cent of cancer cells from patients' bodies, while allowing the salvation of 61 per cent of healthy marrow cells. Marrow is susceptible to damage from chemotherapy or radiation, so some marrow is generally removed before treatment for reinsertion after the treatment is over. But if cancer cells are still in the marrow, the cancer can recur. The new technique uses three monoclonal antibodies attached to magnetic beads 1.5 microns in diameter. Marrow cells are 20-25 microns across. The antibodies attach to tumour cells, and a magnet is then used to remove the antibodies, with the tumour cells still attached. (Extracted from New Scientist, 12 May 1990)

Biodegradable bone implants

Biodegradable bone implants that carry bone growth stimulating proteins in slow release form are being developed in research by Carla P. Desilets et al. at the Army Institute of Dental Research (Washington, DC). Polylactic and polyglycolic acids are being used as the scaffolding and spacers to bridge large gaps in damaged bone. The bone growth protein is microencapsulated in biodegradable polymers. After implanting, bone precursor cells move to the implant site, then develop into solid bone in the presence of the growth protein. The scaffolding eventually deteriorates. For thin, facial bones, the protein is carried directly by the scaffolding material. In thicker bones, the microencapsulated protein is carried by tubes between the bone stumps. In animal tests, new bone growth was found to follow the contours of and had the same strength as the original bone.

In similar research, Massachusetts Institute of Technology (Cambridge) researchers are developing implants using polyanhydride biodegradable plastics. The first commercial application may be an implant that carries slow release antibiotics to treat osteomyelitis. (Extracted from New Scientist, 5 May 1990)

Growth hormone reverses aging, suggest scientists

Genetically engineered human growth hormone (HGH) could reverse some of the effects of aging in elderly men, US researchers claim.

Scientists from the Medical College of Wisconsin and the Veterans Affairs Medical Centres, writing in the New England Journal of Medicine, published results from the first clinical trial of HGH in elderly men.

The results are based on 17 otherwise healthy men aged between 61 and 81 whose secretion of natural growth hormone had stopped. The scientists measured changes in body composition in a placebo controlled trial. According to Dr. Daniel Rudman, author of the paper, "the effects of HGH on body composition were highly significant".

The men were studied for a six month period to confirm their body composition and for an additional six month period exposed to the hormone. During this time, 12 were injected with HGH and the remaining 7 were used as the control group.

HGH increased body weight by an average of 8.8 per cent, reduced the amount of fatty tissue by 14.4 per cent and increased skin thickness by per cent.

Growth hormone therapy has become accepted as a treatment for Turner's Syndrome (a deficiency of natural HGH) in children. It is also used in sufferers of pituitary disease (the endocrine gland that secretes natural growth hormone). HGH has been demonstrated to accelerate growth, but no long-term evidence exists to show the hormone produces long term gain.

Rudman notes that the results can only be extrapolated to apply to one-third of elderly men, but point to a possible treatment for certain illnesses.

However, the use of HGH is not without side-effects. Adverse events have been linked to an increased incidence of hypertension and predisposition to diabetes. There may also be an increased risk of malignancy.

According to informed sources, further work is needed to establish whether HGH should be used into adulthood, that is, if it carries risks that outweigh the benefits and whether it benefits people who do not have a natural HGH deficiency.

Clinical trials of HGH in the elderly are understood to be planned in the UK at St. Thomas' Hospital in London. (Source: European Chemical News, 23 July 1990)

Luminescent reporter for DNA found

A novel transition-metal complex that luminesces upon binding to DNA may be applicable as a sensitive probe for DNA assays, report Jacqueline K. Burton of California Institute of Technology, Nicholas J. Turro of Columbia University, and their colleagues in the US and at Université Louis Pasteur, Strasbourg, France. The group had earlier developed Ru(1,10-phenanthroline)$_3^{2+}$ and its derivatives as spectroscopic probes for DNA structure, but high background luminescence of the free (unbound) form of the complexes, weak binding affinities, and low luminescence enhancement upon binding DNA prevented their broad application as probes. Now the group finds Ru(2,2'-bipyridine)$_2$[dipyrido(3,2-a:2',3'-c)phenazine]$^{2+}$ binds avidly to DNA, displays intense photoluminescence in its presence, and shows no background luminescence in aqueous solution at ambient temperatures. It also responds sensitively to differences in helix structure via changes in both photoluminescence intensity and emission maximum. "We therefore conclude," they write, "that Ru(bpy)$_2$(dppz)$^{2+}$ can serve as a true molecular "light switch" for DNA structures, and tethered onto oligonucleotides, the complex may be useful as a sensitive, non-radioactive, luminescent DNA probe in both heterogeneous and homogeneous assays." (Reprinted with permission from Chemical and Engineering News, p. 23, 25 June 1990. Copyright (1990) American Chemical Society)

Osteoarthritis gene found

US scientists have established a genetic link with the cause of osteoarthritis, according to a paper in the Proceedings of the National Academy of Sciences. Scientists from the Thomas Jefferson University in Philadelphia and the Case Western Reserve University in Cleveland have identified a mutation in the gene encoding collagen II, which appears to be a cause of the disease.

Osteoarthritis is the most common form of arthritis, primarily affecting cartillage, causing it to wear, fray and ulcerate. In the most extreme cases, it can disappear entirely, leaving a bone-on-bone joint. The mutation occurs in the gene encoding collagen II, a protein that strengthens the cartillage that covers tissue and joints and typically breaks down in osteoarthritis.

The researchers found the gene mutation in all nine osteoarthritis sufferers in three generations of a 19-member family. The gene mutation was not found in any unaffected members or in 57 unconnected persons. The reseachers first postulated that a genetic defect correlated to osteoarthritis in a paper published in the New England Journal of Medicine in February.

The discovery offers prospects for developing diagnostics, to identify individuals predisposed to the condition and, with further research, the development of preventative treatments. Current therapies for the condition are generally limited to drugs which relieve pain and inflammation. (Source: European Chemical News, 10 September 1990)

Gene expressed in vivo via direct transfer

A new technique in which recombinant genes are expressed in vivo after their direct introduction to tissue sites could prove useful for the treatment of diseases such as atherosclerosis and cancer, say Elizabeth G. Nabel, Gregory Plautz, and Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor. The same group showed earlier that genes can be expressed in vivo by implanting genetically modified endothelial cells at specific sites. However, this technique requires prior preparation and transduction of the endothelial cells, a process that takes several weeks. The researchers demonstrate expression of a gene in pig arterial tissue after direct transfer of genetic material (instead of previously transduced cells) to the site. When a recombinant β-galactosidase gene is introduced to a site – either by direct infection with a retroviral vector that expresses the enzyme or by transfection with liposomes containing β-galactosidase expression vector plasmids – β-galactosidase activity is detected at the site for weeks or months afterward. (Reprinted with permission from Chemical and Engineering News, 17 September 1990, p. 20. Copyright (1990) American Chemical Society)

Neurogenetic clones brain receptor, migraine-drug template

A newly cloned brain protein has "opened the door on a huge new landscape" in designing drugs for migraine, anxiety, depression and eating disorders, declares Paul R. Hartig, chief of Neurogenetic Corporation, Paramus, N.J.

The company announced that after three years of research it has cloned human serotonin neuroreceptor 5 HT-1D, which scientists believe controls a variety of neurological functions.

Hartig allows that his team cloned the receptor using standard homology and polymerase chain reaction, but he declines to be more specific. Neurogenetic will supply the receptor to drug companies in living mammalian cell cultures or biological membrane preparations. (Extracted from McGraw Hills Biotechnology Newswatch, Vol. 10, No. 13, 2 July 1990)

Genes fired from microcannons take aim at somatic-cell therapy

An enzyme extracted from mouse-ear tissues glowed greenish-yellow for ten days after it had been bombarded by gold microprojectiles payloaded with the gene for luciferase. In surgically exposed mouse liver, the gene coding for the luminescent product – expressed in hepatocytes – lasted half as long.

Duke University molecular biologist Stephen A. Johnston reported the "biolistic" cell

transformation here in June to the 41st annual meeting of the Tissue Culture Association. Using a ballistic device that shoots DNA-coated particles into cells, his experiment demonstrated - visibly - the feasibility of performing somatic gene therapy in vivo, by implanting genes directly in deficient tissues and internal organs.

The gold microspheres average three microns in diameter, and travel a one-centimeter flight path with a velocity of "at least a kilometer per second," says Johnston, "comparable to a high-speed rifle bullet".

His team included members from Cornell University, which originally developed the particle delivery system, and E.I du Pont de Nemours & Co., Wilmington, Del., which manufactures Cornell's gene gun. To penetrate the liver with minimal trauma, the Duke researchers modified the micromissle-launcher, by replacing its gunpowder charge with high-pressure helium as propellant. It also converted the bench-model du Pont apparatus to a portable, hand-held unit, which Duke is patenting.

Transformation of murine ear, liver and skin lasted only a few days, Johnston told his audience here, noting that "Short-lived expression may be desirable in some applications of gene thereapy." In future, more stable plasmids, fitted with integrative, replicative or infectious sequences, would permit more permanent therapies or transoenic effects, he pointed out. (Source: McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

Research on animal genes

Insect produces fish proteins

Two Atlantic wolffish antifreeze proteins were produced by transgenic Drosophila melanogaster in research by Derrick E. Rancourt and colleagues at Queen's University (Kingston, Ontario). The genes for the two proteins were transplanted by P-element tranformation into flightless Drosophila to the yolk polypeptide gene promoters. The female insects produced the biologically active fish antifreeze proteins at concentrations of 1.5-5 mg/ml. The protein was purified from the insect hemolymph accumulation site by thermal denaturation, followed by step elution from SP Sephadex and reverse-phase HPLC. The research demonstrated that high yields of foreign proteins can be otained through transgenic Drosophila, which are relatively inexpensive to maintain even on a laboratory scale. (Extracted from Bio/Technology, May, 1990)

Mice produce human urokinase protein in milk

Mice, genetically altered with the human urokinase gene, lactated 1-2 mg of human urokinase protein/ml of mouse milk, in research by Harry Meade et al at Biogen (Cambridge, MA). The mice received a hybrid bovine alpha-sub s-1 casein/human urokinase gene. The recombined gene is specifically targeted to produce human urokinase protein in lactating tissue, rather than in the kidney, as normal. Other than the protein production in the milk, the mice showed no other differences. Their litters were normal-sized and healthy. Future research may apply the transgenic techniques to farm animals for greater production of recombinant products. For example, a transgenic cow could produce more than 20 g of protein/5 gal of milk/day. (Extracted from Bio/Technology, May 1990)

Biologists pioneer "reverse genetics"

The fruit fly Drosophila melanogaster has contributed more to our understanding of the mechanics of inheritance, and of genes themselves, than any other multicellular organism. Traditionally, biologists have been able to identify genes that code for proteins that are important to the function of the fly only after observing how specific mutations affect the appearance or behaviour of the fly. But it has been much more difficult to move in the other direction - to work out what a gene that has been identified actually does in the fly. This approach is known as "reverse genetics".

Two teams of researchers working independently at the University of Glasgow and the California Institute of Technology (Caltech) in Pasadena have pioneered the "reverse genetics" approach. Their work will help biologists to discover the function of the growing number of genes which have been cloned directly without any prior knowledge of their role in the biology of the fly. Their approach may also prove to be applicable to other organisms, as diverse as worms and plants.

Both groups exploited the mutagenic properties of "transposable elements", pieces of DNA that have the ability to move from place to place within Drosophila chromosomes. To a first approximation, transposable elements insert themselves randomly into new chromosomal sites. They may produce a mutation if they insert themselves into a gene. This mutation will manifest itself as a defect in some aspect of the biology of the fly.

The reseachers used polymerase chain reaction (PCR), originally devised by a group at the Cetus Corporation in the US. PCR allows scientists to selectively amplify a specific region of DNA in a few hours.

Using the technique, the groups at Glasgow and Caltech were able to detect transposable elements when they inserted themselves close to specific cloned Drosophila genes.

The scientists designed one primer that was unique to their gene of interest - the "gene specific primer" - and a second primer that was unique to the transposable element. In this way, exponential amplification can occur only if a transposable element inserts itself in, or near to, the gene of interest. The problem is that the desired mutation will be rare within a population of flies in which mutations had been induced.

The Glasgow team found an efficient solution to this problem. They took batches of 1,000 females in which transposable elements had moved around and allowed them to lay eggs. The team isolated DNA from the eggs and used PCR to determine whether at least one female within a batch was laying eggs with a transposable element close to or within the gene of interest. If this was the case, the researchers divided the batch of females into groups of 100, and repeated the process.

By a process of elimination, the team was able to isolate the rare female laying the "golden egg". Happily, the golden eggs also grew up to be flies with the expected genetic defect.

Both groups are interested in genes expressed in the fly brain. Many such genes exist but little

is known about their function. The new technique will make it easier to isolate mutations in brain-specific genes.

Because the polymerase chain reaction is limited only by the imagination of the researchers applying it, it could be used in the context of organisms as diverse as worms and plants i the future. (Source: New Scientist, 28 July 1990)

Virus insecticides a step closer

Scientists at the Division of Entomology in Canberra (Australia) have recently made several major advances in the development of genetically engineered virus insecticides to control Heliothis armigera, the cotton bollworm. As a result, a joint venture has been set up between the Division, ICI (Australia) and ICI (UK). The first three years' work will be carried out by the Division with subsequent development being taken up by ICI.

Heliothis is one of the world's major insect pests and infests a large number of crops, particularly cotton, oilseeds, grain legumes and many varieties of vegetables. This damage occurs throughout the world and its annual cost in Australia alone is estimated to be $450 million. As Heliothis and other pest species develop resistance to chemical insecticides and public concern increases over the widespread use of these insecticides, there is a pressing need to develop safe alternative means of control.

The Division of Entomology team, led by Dr. Peter Christian, has identified several genes which will make a normally slow-acting virus highly virulent. They are now attempting to clone these newly identified genes into a species-specific virus.

The selected virus, known as the Nuclear Polyhedrosis Virus (NPV), is from a strain specific to larvae of Heliothis moths and was originally isolated from Heliothis armigera in Queensland. The virus is highly infectious but it usually takes about a week to kill larvae in the field. For high value crops such as cotton this is far too long, as the larvae can do considerable damage to the crop before the virus kills them.

Dr. Christian says that the team hope eventually to engineer the Australian NPV to act about five times faster than its naturally occurring parent strain. To do this, they hope to clone genes from other insect species and insect pathogens that will allow the virus to manufacture an insecticidal protein that will kill the pest within 24 hours. In effect, the virus will act as the delivery system for the insecticidal protein.

Another option for engineering the virus is to insert some of the Heliothis's own genes into it. The expression of these genes at high levels or at an inappropriate time in the development of the larvae will also make the NPV more lethal.

Natural isolates of the NPV have been obtained and mass produced in Heliothis armigera caterpillars and the cloning, mapping and manipulation of the virus' genome is now under way. To ensure the safety of the system, the insecticidal proteins finally chosen will be specific to Heliothis.

This CSIRO/ICI project is also supported by the Australian Cotton Research Council, the Grain Legumes Research Council and the Oilseeds Research Council. For more information contact:
Dr. Peter Christian, CSIRO Division of Entomology, GPO Box 1700, Canberra, ACT 2601, Australia or Dr. Anne Campbell, ICI Australia Research Group, Newsom Street, Ascot Vale, Vic. 3032, Australia. (Source: CSIRO Industrial Research News, No. 199, April 1990)

Research on plant genes

Plans set for mapping plant genome

NSF will co-ordinate efforts to map the complete genome of the simple weed Arabidopsis thaliana. The agency concluded an agreement with the National Center for Human Genome Research, part of NIH; the Office of the Assistant Secretary for Science & Education of USDA; and the Department of Energy's Office of Energy Research. NSF points out that many researchers have already adopted the weed as a model for studying plant biochemistry, genetics, and physiology. Arabidopsis undergoes the same processes of growth, development, flowering, and reproduction as other higher plants, including important crop plants. But a complete set of Arabidopsis genes - the genome - has about 30 times less DNA than does a corn genome or a human genome. Adding to its popularity as a model, the weed is small, a prodigious seed producer, and short lived. It can be genetically engineered to incorporate the genes for economically important products made by other plants. NSF says it has been working with the Arabidopsis research community to develop long-term strategies, to be completed this summer, for mapping and sequencing the genome. (Reprinted with permission from Chemical and Engineering News, 18 June 1990, p. 18. Copyright (1990) American Chemical Society)

Monitoring gene transfer in plants

Transference of genes into plant cells might be monitored more easily with a new gene that makes the cells red, according to researchers at the University of Georgia (Athens) and Hi-Bred International (Johnston, IA). The gene Lc, isolated from maize, and modified so that it is always active, can be inserted into cells along with the desired gene(s), so that cells that take up the foreign DNA will make the red pigment anthocyanin. The red is obvious, so no further biochemical tests are needed to determine if a cell has taken up the foreign DNA. (Extracted from New Scientist, 5 May 1990)

Research on viral genes

Genetic probes sort out the good wood from bad

The oil company Shell has revealed that it has just begun pioneering experiments with new genetic techniques that could overcome one of the major problems facing forestry researchers - the time it takes for trees to grow. The technique could enable reseachers to identify strains of trees with desirable characteristics - such as uniform wood quality - at the seedling stage, saving them from waiting years for trees to mature and such characteristics to become apparent naturally.

Reseachers at Shell's Sittingbourne Research Centre in Kent are attempting to apply so-called DNA-probe technology to the selection of tree seedlings.

The technology has already been applied in medical diagnosis (to trace genetic diseases such as muscular dystrophy, for example) and in research on agricultural crops such as maize and tomatoes. However, the researchers at Shell claim to be among the first to apply the techniques to trees.

They have already had some success in improving existing techniques for propagating trees using cuttings. By growing the cuttings - which proliferate on stumps of felled trees - foresters are able to identify trees with desirable characteristics.

Looking further ahead, Shell is evaluating the possibilities of "embryogenesis", the growth of cloned cells from trees in fermenters. The aim is to induce individual cells to grow into embryos that could then be germinated and grown like any other trees.

The company moved into forestry because it identified a world-wide shortage in timber supply but the first goal is to develop "short-fibre, high-quality pulps for high-quality paper". In aiming for this and other specialist markets, the company is keen to exert more control over the uniformity of the wood produced and the means to vary its properties to suit specific uses.

Shell has built up its forestry operations in New Zealand, Chile, Brazil, the Congo and South Africa, mainly in eucalpytus and tropical pine. (Extracted from New Scientist, 28 July 1990)

Rice chromosome mapped

Japan's National Institute of Agrobiological Resources (Ministry of Agriculture, Forestry and Fisheries), with the co-operation of the Faculty of Science of Toho University, has completed a chromosome map of rice for the first time in the world. This is a very important milestone in the history of genetic studies on rice. Since the 12 pairs of chromosomes shrink down to 1-2 microns at metaphase, it was difficult to identify a single chromosome or to discriminate one from another. The research group studied preshrinkage chromosomes and degrees of partial shrinkage in detail using an image analyzer they developed. In this way, they marked 4-10 addresses in each chromosome. This is expected to be a landmark for locating rice genes precisely. (Source: Bio/Technology, Vol. 8, May 1990)

Rice improvement

Professor Uwozumi's group at Tokyo University, Department of Agriculture, has engineered Klebsiella oxytoca, a bacterium associated with rice, for improved nitrogen-fixing abilities. Normally, K. oxytoca is much poorer at fixing nitrogen than Rhizobium, which forms root nodules on beans. Uwozumi and his colleagues have spent the last 10 years analyzing K. oxytocas's 20 nitrogen fixation-related genes. From these, they have isolated two major ones - the gene for suppressing nitrogen fixation when ammonia is present in the soil, and the gene for promoting fixation. The group successfully destroyed the suppressor gene, thus enhancing the promoter's function. When they added the engineered microbe to soil, they found that the rice increased in nitrogen content and dry weight by about 30 per cent. The nitrogen content of the soil increased also. The engineered microbe is also genetically stable.

Researchers at the National Institute of Agrobiological Resources and Mitsui Toatsu Chemicals (Tokyo) have successfully identified and isolated a rice promoter gene, which regulates the expression of foreign genes. The promoter has 850 base pairs and enhances gene expression 10-fold, specifically in rice leaves. The group expects to develop a new variety that is insect- and herbicide-resistant. (Source: Bio/Technology, Vol. 8, June 1990)

Experiments to improve American cotton

Nisshinbo Industries (Tokyo), in co-operation with Texas A&M University (College Station, TX), has started experiments to improve an American variety of cotton. Cotton varieties are usually adapted to local climatic conditions and cannot be grown in other areas. This leads to the present monopoly in the supply of raw, good quality cotton.

The researchers intend to incorporate the genes of the top quality Carribean and Egyptian varieties into an American variety. Texas A&M will hold possible patents and Nisshinbo will have priority for obtaining a license. The cotton will be grown on American cotton farms and marketed under the Nisshinbo trade-name. (Source: Bio/Technology, Vol. 8, May 1990)

Genetically engineered wheat

Research scientists at the Research Institute of Agricultural Resources of Ishikawa Agricultural College, collaborating with a group from Kyoto University's Research Center for Cell and Tissue Culture, have successfully engineered an exogenous gene into wheat. To date, genetically engineering wheat has been very difficult with conventional methods, including using Agrobacterium's Ti plasmid. The researchers (from Professors Shimada and Morikawa's laboratories) introduced the exogenous gene into wheat cells derived from pollen embryos via a particle gun. The genetically engineered wheat has grown to five centimeters and should mature within two months. (Source: Bio/Technology, Vol. 8, May 1990)

Foreign gene introduced into rape

Researchers at Tokyo's Plant Research Institute, a joint venture of Mitsubishi and Mitsubishi Kasei, have successfully introduced a foreign gene into rape using electroporation. They prepared rape protoplasts from germ-free hypocotyl cells and introduced an exogenous glucuronidase gene together with a hygromycin-tolerance marker gene. Then the protoplasts were "nurse-cultured" on cultured tobacco cells and screened by hygromycin, resulting in three fertile individuals with the foreign gene. Although electroporation has been widely adopted for plant transformation as a highly efficient gene introduction method, the present result is the first success in rape, establishing a practical method for developing genetically engineered rape variants. The company is searching for target genes for transformation; researchers expect to develop F1 progeny with higher fat content or improved lipid constituents within three years. (Source: Bio/Technology, Vol. 8, August 1990)

Gene-spliced corn heralds customized crops

Genetic engineers at Ciba-Geigy, the Swiss pharmaceuticals giant, claim to have modified the genes of a commercial variety of corn seed. This takes biotechnology closer to one of its Holy Grails - genetically altered cereal crops that can resist pests, diseases and harsh environments.

Biotechnologists working in agriculture have already altered the genetic characteristics of so-called broad-leaved crops such as soya beans, tomatoes and tobacco plants. They have found it far more difficult to modify cereal crops, such as corn and wheat, which are so important to the world's food supply.

The company made its breakthrough using so-called "biolistic" technology. This involves

firing tiny metal projectiles coated with DNA into the heart of the plant's cells in culture. The projectiles travel at extremely high speed from a small gun operated with gunpowder cartridges.

In the past it has proved difficult to reach the protoplast of the cells of cereals, since they do not respond to the bacteria that plant technologists normally use to penetrate the cell walls of broad-leaved crops. This has forced genetic engineers to strip the cereal cells of their thick, protective outer wall in order to reach active genetic material - a process which lowers the success rate.

Although the biolistic approach has simplified the breeding process, the conditions under which the company nurtures its genetically engineered seeds, such as the media in which they grow and the temperature of the surroundings, are just as important. Researchers still rely on a trial and error approach, whereby the skill of picking the plant cells in culture that look likely to grow successfully (and express the foreign genes) can be paramount.

The company has produced a first generation of plants from its altered seeds, and is waiting to see whether these will produce their own seeds and a subsequent generation of plants that carry the alien genes.

As yet the company has inserted only passive marker genes into the corn. It must now identify the combination of active genes and DNA to control them that is best suited to the plants. However, getting any foreign DNA at all expressed in these fully grown plants is by far the most awkward step. (Source: New Scientist, 1 September 1990)

PAP gene cloned

The UK firm, Agricultural Genetics Company (AGC) has successfully cloned the gene which encodes pokeweed antiviral protein (PAP). Recently, a monoclonal antibody-PAP adjunct molecule was reported to have promising therapeutic activity against the AIDS virus. AGC's aim is to introduce PAP genes into other plants to impart a viral resistance. However, the cloning of the gene in E. coli bacteria, could allow useful quantities of PAP to be produced without the need to extract it from the pokeweed plant, the company said. (Source: European Chemical News, 1 October 1990)

Molecule of the decade

In an elegant integration of biochemistry and genetics, researchers are closing in on what may be the first "real" plant hormone. Professor Andy Johnston, of the University of East Anglia, draws attention to the recent identification of what he considers "one of the most exciting molecules of the decade".

Using the alfalfa symbiant Rhizobium meliloti, scientists in France have proposed a structure for the active molecule which induces root hair curling, a crucial first step for the formation of nitrogen-fixing nodules in legumes.

The root hair curling factor, now identified as a tetraglucosamine, induces morphological changes in the host plant at concentrations as low as 10^{-13}M. It is more active than "classical" plant hormones, such as auxins and cyotkinins, by a factor of millions.

Several rhizobium genes have been sequenced and correlated with existing data bank maps, but it has not been possible to "ascribe precise functions to individual genes", according to Johnston. However, this exercise in deduction may be vindicated by the work of biochemists. As it turns out, the computer analysis predicted four gene products which are indeed key components of the root hair curling factor (see table).

Gene mapping predictions

Gene	Deduced product
nodM	Protein similar to glucosamine synthase
nodL	Acetylase
nodF	Lipid moeity synthesis
nodE	Lipid moeity synthesis

The factor may have an important practical use, Johnston suggests. In North America, for example, soybean cultivation is entirely dependent on soil inoculation with foreign rhizobium strains, as soybean symbiants are not indigenous. "One of the severe problems of introducing just pure rhizobium is to get the little blighters to nodulise", he says. Introducing the factor might increase the newcomer's chances of competing with other soil bacteria. (Source: Chemistry & Industry, 3 September 1990)

Plant "soap" enzyme isolated

Calgene Inc. has announced it has discovered a "new enzyme" implicated in the synthesis of laurate, a key fatty-acid component of soaps, detergents and lubricants, usually found only in tropical-plant oils. Calgene's objective, says senior scientist Maelor Davies, is to clone the genes responsible for this synthesizing enzyme and introduce them into commercial rapeseed. (Source: McGraw-Hill's Biotechnology Newswatch, 16 July 1990)

Scientists report AIDS inhibition

US scientists have discovered a class of compounds which block the infection of cells by the human immunodeficiency virus type 1, HIV-1, in laboratory studies.

Infection by HIV-1 is initiated when its envelope protein (gp 120) binds to a receptor, the cell surface glycoprotein, CD4. Robert W. Finberg and co-authors report that this process can be blocked by a group of small molecules, N-carbomethoxycarbonyl-prolyl-phenylalanyl benzyl esters (CPFs).

CPFs may be important candidate drugs for the treatment of AIDS according to the scientists. Clinically, the compounds are expected to inhibit HIV infection and to reverse any immunosuppression or toxicity due to soluble gp 120.

The paper highlights the potential therapeutic importance of one of the compounds, CPF (DD), which prevents the spread of HIV-1 from a small number of infected cells to a larger population of uninfected cells.

The research was carried out at Harvard Medical School's Dana-Farber Cancer Institute and Department of Chemistry.

Scientists from the Roche Institute for Molecular Biology in Nutley, New Jersey, reported the identification of a human cellular protein that

interacts with the HIV Tat protein. The finding could lead to a new approach to blocking HIV replication in cells.

The researchers describe a compound termed Tat-binding protein 1 (TBP-1), found by screening a cloned DNA library with Tat, for a protein with suppression activity.

The Tat protein is one of three HIV proteins which regulate the replication of the virus in an infected cell, the others being termed rev and nef. It is believed that Tat and rev proteins are essential for the replication of the HIV.

The Roche scientists speculate that TBP-1 may be one of many cellular factors which modulate Tat function, and that an understanding of the mechanism could provide a means of controlling the process, leading to a possible way of blocking transactivation at the cellular level. (Source: European Chemical News), 30 July 1990)

Research instrumentation

NMR sheds light on DNA complexes

A new solid-state nuclear magnetic resonance technique makes it possible to determine the orientation of atoms and functional groups in DNA intercalation complexes, according to Pei Tang, Chi-Long Juang, and Gerard S. Harbison of the State University of New York, Stony Brook. A wide range of mutagens, drugs, and carcinogens can bind with DNA, but it has been unclear how DNA changes structurally to accommodate intercalators, a process in which the interbase spacing can essentially double. Using the NMR technique to determine the structure of a complex of proflavine with fibrous DNA, the researchers detected major changes in the orientation of phosphodiester groups upon proflavine binding but found no changes in the puckering of the deoxyribose ring, a mechanism that had been proposed earlier. The technique, they say, could be "of immense value in determining the structure and dynamics both of drugs bound to DNA and of the DNA itself". (Reprinted with permission from Chemical & Engineering News, p. 31, 19 July 1990. Copyright (1990), American Chemical Society)

Gene gun transforms animal cells in vivo

Researchers at Duke University have developed a simple yet versatile method of inserting novel genes into the somatic cells of live animals: shoot them in with a biological version of an air-rifle. Conventional methods for transforming such cells are complex and indirect. The cells targeted to be transformed are drawn out of the organism, transformed with a retrovirus and then reintroduced. The US National Institutes of Health researchers plan to use this approach with bone marrow cells to correct bone marrow deaminase deficiency in a select group of patients.

The original gene gun used a gunpowder-like explosion to shoot the DNA into the target cells, which can result in damage to the more delicate animal cells. The new design uses high-pressure gas instead. The redesigned gun - also called a "biolistics device" - can insert novel genes into the ear, skin and surgically exposed liver cells of mice. (Source: Biotechnology Bulletin, Vol. 2, No. 6, July 1990)

Undercooling

A new biopreservation technique, developed at the University of Cambridge in eastern England, may solve difficulties associated with storing active biological proteins and cell cultures. Botanist Felix Franks invented undercooling, which avoids ice formation by suspending biological matter in a carrier fluid, stored at an average of -20° C. To recover activity, suspensions are simply warmed to ambient temperature. The system is expected to be used for biotechnology's intermediate products, as well as for blood components and cells. (Source: Canadian Laboratory, Vol. 1, No. 3, January 1990)

Setting the standard for determining capacity

Most manufacturers of affinity gels and membranes calculate and state capacities in unrealistic ways. They test the affinity media by overloading the theoretical capacity of the gel or membrane by two or three fold with antibody, wash, then elute the bound antibody to determine the "aimed capacity. This method boosts the capacity rating to reduce the $/mg capacity value that you calculate when determining the most economical product to use. However, setting capacity values using this method is misleading because under practical experimental conditions, you will not be able to attain these high capacities. That is why most manufacturers recommend that you operate at 50-75 per cent of the stated capacity, which leaves you wondering if you get what you paid for.

When NYGene states capacity values, it means the amount of antibody that you can challenge a MASS^R device with and recover 85-95 per cent on a single pass and expect purity of >95 per cent. If you try this with other affinity gels or membranes, you will find that they overstate recovery capacities by a factor of 2 to 3 or more.

It is important to note that NYGene and most manufacturers use polyclonal antibodies to test products; monoclonal are too expensive and variable to use in quality control. You will see some variation between species and subtypes especially monoclonals. Therefore, refer to the table below to review anticipated capacities with NYGene MASS devices.

Species	Subclass	Amount of Purified Ab by 1 mg MASS Protein g Devices	10 mg
Bovine	IgG	1.4 mg	11.2 mg
Goat	IgG	0.8 mg	5.9 mg
Human	IgG	1.2 mg	9.1 mg
Mouse	IgG_1	1.3 mg	10.0 mg
Rabbit	IgG	1.3 mg	9.5 mg
Rat	IgG_{2a}	0.8 mg	5.9 mg

(Source: Company Press Release, 10 October 1990)

General

2-D NMR applied to RNA

Using two-dimensional nuclear magnetic resonance methods originally developed for studying protein structure, chemists at the University of California, Berkeley, have determined the precise structure of an unusually stable, four-nucleotide ribonucleic acid loop. Chemistry professor Ignacio Tinoco Jr., Chaejoon Cheong and Gabriele Varani probed the very stable and common RNA hairpin 5'GGAC(UUCG)GUCC3' (loop nucleotides in parenthesis), which forms when the polynucleotide chain folds back on itself to form a short, intramolecular base-paired helix (5'GGAC pairs with GUCC3') called the stem. Among their findings, they discovered that the loop is stabilized by a G-U base pair, with the guanine in the syn conformation.

between the first and last nucleotide residues in the loop, effectively making a loop of only two residues. They also showed that the second uracil residue in the loop, which is susceptible to chemical modification, protrudes into solution, while the cytosine residue is stacked inside the loop and makes an internal hydrogen bond with a phosphate group. (Reprinted with permission from Chemical and Engineering News, 3 September 1990, p. 28. Copyright (1990) American Chemical Society)

Enzyme-Like catalysts move ahead

As part of the long-term scientific effort to design and synthesize catalysts that resemble natural enzymes, scientists from the Univeristy of Colorado Medical School (Denver) report designing and assembling a protein with enzyme-like catalytic activity. The researchers based the protein design on the digestive enzyme chymotrypsin. They built a 75 amino-acid structure from a functional "triad" of three amino acids found in the enzyme, using computer-aided design techniques. When they synthesized the relatively simple design, they found it reacted the same way and with the same specific target molecules as the natural enzyme. (Source: Chemical Week, 4/11 July, 1990)

Research into low molecular weight compounds

Cortex Pharmaceutica s, a company based in Irvine, California has received a $45,000 federal small business grant for innovative research into low molecular weight neural growth factor mimics. Such low molecular weight compounds are research targets because they are able to cross the blood-brain barrier more easily than larger molecules. If successful, the programme could attract up to $500,000 in federal funds. (Source: European Chemical News, 24 September 1990)

French group prepares copper complexes that resemble "inside-out" DNA

Copper complexes that mimic the double-helical structure of DNA but turn the structure inside out have been prepared for the first time by chemists at Louis Pasteur University in Strasbourg, France. The new molecules, while not themselves DNA, may enable scientists to gain insights into how it interacts with other molecules. Such studies could eventually lead to the development of new drugs that target DNA.

The new molecules, prepared by Professor Jean-Marie Lehn and co-workers Ulrich Koert and Margaret M. Harding, consist of an organic "spiral staircase" curling around a column of three or five copper ions fashioned from two intertwined strands of a linear oligomer containing bipyridine units. Attached to each bipyridine unit and flaring outward is a pair of deoxynucleosides, such as deoxythymidine.

In Lehn's molecules, by contrast, the deoxynucleosides are outside the helix and the stacking is between pyridines. The overall charge, due to the copper ions, is positive.

Remarkably, the nucleohelicates assemble spontaneously when the bipyridine chains are allowed to interact with copper ions. Scientists are not sure what drives this spontaneous assembly, although an important controlling factor could be the stacking of coplanar pyridine rings.

Lehn has been studying such self-assembling helicates for years, but the new twist is the addition of nucleoside appendages. He says other groups can also be attached to the helix, such as amino acids, sugars, and electroactive and photoactive units. (Abstracted with permission from Chemical and Engineering News, 30 July 1990, p. 7. Copyright (1990) American Chemical Society)

Antibodies bind metal ions

In yet another step towards realizing the full potential of catalytic antibodies, scientists at the Research Institute of Scripps Clinic, La Jolla, California, have produced antibodies that bind copper, zinc, and cadmium ions. Scripps chemists Brent L. Iverson and Sheila A. Iverson and colleagues including Stephen J. Benkovic and Richard A. Lerner modified the gene that encodes the light chain of a well-characterized antibody to incorporate three histidine residues in the chain. The positions of the histidines were based on the positions of three histidine residues in the zinc-binding protein carbonic anhydrase B. Using a technique developed at Scripps for recombining antibody light- and heavy-chain fragments, the scientists produced antibodies that contain a metal ion binding site. Although the metal binding constants for carbonic anhydrase B are a factor of about 1 million greater than those estimated for the modified antibody, the scientists suggest that the antibody can be saturated with metal at metal concentrations that should be compatible with catalytic antibody applications. (Reprinted with permission from Chemical and Engineering News, 13 August 1990, p. 30. Copyright (1990) American Chemical Society)

"DNA fingerprinting" standards needed

"Immediate attention" should be given to setting standards for using DNA profiles to identify individuals, says a report issued by Congress Office of Technology Assessment. Nevertheless, OTA affirms that DNA tests are reliable and valid when performed properly.

Use of the technique commonly known as "DNA fingerprinting" in criminal investigations has mushroomed: OTA estimates it has been used in more than 2,000 criminal cases since being introduced to the US in 1986. The tests are used even more often in paternity disputes.

In more and more cases, however, defense attorneys have successfully challenged DNA evidence in court by uncovering sloppy procedures and subjective interpretations. Scientists, too have warned about the lack of rigorous standards.

Currently, OTA points out, there are no standards as to how the tests should be performed and interpreted, and no agreement among forensic scientists, researchers, and law enforcement officials over who should set such standards. Consensus must be reached on issues such as the proper reagents and gel controls, electrophoresis conditions, rules for matching DNA banding patterns, and the population data necessary to compute the likelihood of coincidental matches.

The criteria necessary to declare a match are critical, says the report, because "band-shifting" often occurs - that is, two samples may show similar banding patterns slightly offset from one another. Whether or not to call such shifted bands a match can be crucial to determining a subject's guilt or innocence.

Defense attorneys have recently raised the question of whether mariages within ethnic groups make it more likely that two samples match by chance

than the odds of roughly one in a million often cited by proponents of DNA fingerprinting. This is one focus of attention of a National Research Council panel that is preparing a report on DNA profiling.

In addition to standards and quality assurance, it explores privacy issues raised by proposals by law-enforcement officials to set up DNA data banks to help track suspects and identify repeat offenders. (Abstracted with permission from Chemical and Engineering News, 13 August 1990, p. 6. Copyright (1990) American Chemical Society)

Genome mappers test their system

More than 140 geneticists from around the world met at the University of Oxford in September 1990 to update the map of the human genome and test their data base. The workshop is crucial for the success of a major meeting on the genome which takes place in London in 1991.

Ian Craig, a geneticist at the University of Oxford and the chairman of the workshop's organising committee, said before the start that the computer network set up for the meeting in a traditional college building appeared to be "fully functional".

The existing data base comes from Johns Hopkins University in the US. After the workshop, the newly structured data base will be available online all over the world. Eventually it will handle information on some 100,000 genes.

Each chromosome has its own committee of geneticists. In addition, there is a DNA committee, whose functions include listing all the available DNA probes. It is this committee that will depend more than all the others on using the network successfully because so much of its information will have to be cross-referenced.

The chromosomes generating the most interest at present are X, 5, 6, 19 and 21, although there is no shortage of laboratories interested in working on all sections of the project. (Source: New Scientist, 8 September 1990)

Building artificial chromosomes in yeast

In the three years since the yeast artificial chromosome (YAC) technique hit the pages of the scientific press, the business of genome mapping has been transformed. At a stroke, the technique allows mappers to march in giant leaps along the genome.

In the pre-YAC era, mappers were limited to using DNA fragments up to about 50,000 bases in length. These fragments - cosmids - had several benefits. First, because the cosmids could be replicated in the bacterium Escherichia coli, large quantities could be obtained through the standard process of cloning. In addition, if a DNA sequence of interest was known to be located on a particular cosmid, it was not difficult to pin down: there would be only 50,000 bases to look through.

Cosmids have disadvantages, however, principally because they are smaller than even the smallest genome, not to mention the 3-billion-base genome of humans. Trying to map the human genome with cosmids would be tedious. As with jigsaws, they are always easier if you have larger pieces. That is what YACs give you. The YACs provide jigsaw pieces varying from 250,000 to a million bases, a significant improvement on cosmids.

The size limit of cosmids is determined solely by how large a piece of foreign DNA can be inserted into the vector, which is then introduced into E. coli to be processed. The jump in size allowed by YACs is achieved because the vector is so much bigger, and this can be handled by yeast. Yeast chromosomes vary in size from 250,000 to a couple of million bases. What the YAC technique does is take a large piece of DNA and disguise it as a yeast chromosome. Simply introduce the YAC into the yeast cell, and the cell's molecular machinery will replicate it just like genuine chromosomes.

Like most good ideas, it sounds deceptively easy, but it took the genius of Maynard Olson of Washington University to make it a reality. There are three basic elements of the necessary disguise. Two of them are fundamental components of all chromosome architecture, a centromere (for the centre), and two telomeres (for the ends). The third element is called the ARS, or autonomous replicating sequences, which is necessary to the initiation of DNA replication. These elements had been well characterized by yeast researchers over the years, and so could be pulled off the shelf. The centromere, telomeres, and ARS form the basis of the DNA carrier system, the vector.

One or two other elements were also necessary for the YAC technique to work well, developed in Olson's laboratory by graduate students David Burke and Georges Carle. To introduce the foreign DNA into the YAC, you have to split the vector in two, giving a right arm and a left arm. Mix the foreign DNA with split vectors, and attach the two arms to the ends of the DNA fragment. You have to be certain, of course, that the YAC has a right arm and a left arm, not two right or two left. So, Olson and his colleagues fixed this by adding a gene to each arm, say gene A on the right arm and gene B on the left arm. The trick is that the strain of yeast used will grow only if genes A and B have been introduced together on the YAC. Any other combination, and the yeast simply refuses to grow. It is a form of selectivity well known to geneticists. Other genes are often present on the YACs, to allow variations on this theme of selectivity.

Having fooled the yeast cell that it has got an extra chromosome, and having provided the missing genes necessary for growth, DNA replication can get under way. Although the yeast cell system can handle large pieces of foreign DNA, it cannot be fooled into producing huge quantities of this DNA to the exclusion of its own genome, as can be done with the bacterial system. So the YACs impose limits on how much DNA can be produced, and there are more problems with separating the YAC DNA from the yeast chromosomes. Nevertheless, the large size of the foreign DNA fragments that can be cloned makes it worthwhile.

In addition, because yeast is a eukaryotic cell - a cell with a nucleus - it has less problem replicating certain difficult sections of DNA from other eukaryotic species. In many cases, E. coli appears unable to replicate all of a eukaryotic genome, leaving unavoidable holes in any gene map.

The ultimate aim with genome mapping is to have a series of overlapping YACs that represent the entire genome from end to end. The Medical Research Council Laboratory of Molecular Biology, Cambridge/Washington University, St. Louis, Missouri collaboration is reaching this goal. At this point

it is possible to lay out the YACs in order as a grid, a physical representation of the entire genome sequence. In the case of the nematode, more than 95 per cent of the genome is covered by some 950 YACs (selected from several thousand). These YACs can be laid out over a single piece of filter paper, the size of a postcard, making it the largest organized collection of YACs in existence. The actual number of YACs here is dwarfed by the St. Louis collection from the human genome - 70,000 - but these are not yet organized as sequences in the genome. (Source: New Scientist, 25 August 1990)

DNA sequencing made simple

The human genome initiative relies on the ability to sequence quickly, accurately and cheaply. One of the central goals of the MRC, Cambridge/Washington University, St. Louis collaboration is to put current sequencing technology to the test. The basic method of much modern sequencing remains the same as the one that earned Fred Sanger at Cambridge a share of the 1980 Nobel Prize for Chemistry. Recent modification have focused on ways of automating the process.

In a short DNA strand of 500 bases, the usual size of strand worked with, you need to identify each base, from position 1 through to position 500. The sanger method for doing this actually works backwards. You take single-stranded DNA, and make the complementary strand. This second strand gives you the required information.

A short primer is added to the single-stranded DNA, and this is then bathed in a pool of the four bases - adenosine, thymidine, cytosine, and guanosine - under correct enzymic conditions for growing the second strand. But in the synthesis a small proportion of one of the bases is in a form - the di-deoxy form - that halts chain elongation. Because of the mix of normal to aberrant forms of this base in the reaction, chains may grow only a short way before being stopped by the di-deoxy base, sometimes proceeding all the way to the end, sometimes stopping in between.

With the correct mixture of reagents, the finished pot will contain a mixture of chains, with chain elongation halted at every position in the DNA strand that this particular base occurs. If the base concerned here is adenosine, for exammple, then the reaction pinpoints all the positions in the DNA strand at which adenosine appears.

Run the DNA products of the reaction mixture down a gel that separates chains out according to their length, and you will find a ladder-like separation of the different elongated chains. If you find strands at positions that correspond to lengths of, say, 3, 5, 6, 9, 12 and so on, you know that these are the positions at which adenosine appears in the DNA. Do this same reaction separately for the other three bases, and you can read off the positions of all four bases throughout the DNA strand. That is the sequence.

A key part of this operation, of course, is being able to detect the position of the chains of different length on the gel. Until recently, this was done using radioactivity to tag the chains. The gel was exposed to a piece of photographic film, and the four ladder patterns read off from there. Four years ago, Leroy Hood, of the California Institute of Technology, introduced the idea of using fluorescent dyes to tag the chains, an approach that many believe now leads the field. Fluorescent dyes allow band detection by laser, a trick that could become both accurate and automated. Reliable computerized data collection is a must for the genome project.

The Cambridge and St. Louis laboratories will be testing two automated sequencers, both based on the fluorescent dye approach. One is that invented by Lee Hood and developed commercially by Applied Biosystems Incorporated, California. Wilhelm Ansorge of the European Molecular Biology Laboratory, Heidelberg, devised the second, and this is sold by Pharmacia, Sweden. In the ABI machine, four different fluorescent dyes are used, one for each base. The reaction products are then mixed together, and passed down the gel in a single lane, not in the four lanes required when a radioactive tag is used. A laser detector scans the four colours as the DNA comes down the gel, and records the sequence automatically. The Pharmacia machine uses only one fluorescent dye, and it keeps the products of the four different reactions separate. Four lanes on the gel have to be run for each DNA, as in the traditional radioactive method. But the advantage is that the laser detector is tuned to a single colour, and, in principle, can be more robust than Hood's technique.

Each of the two nematode laboratories will have an ABI machine and a Pharmacia machine, allowing parallel testing under different conditions. Because the ABI machine needs just one gel lane for each DNA to be sequenced, it can run more clones simultaneously than the Pharmacia machine, which needs four gel lanes for each clone. A total of 24 clones (of about 500 bases each) can be run simultaneously on the ABI machine, giving total sequence collection of 12,000 bases in 10 hours. Although the Pharmacia machine can run only 10 clones at a time, it is faster, completing a run in five hours. Theoretically, it could sequence 10,000 bases in 10 hours, but squeezing two runs in for the ABI's one would be pushing the machine and personnel to the limit.

Because the Cambridge and St. Louis teams have the physical map in hand, they will be able to approach sequencing in a targeted way. Specific regions will be parcelled out between the two laboratories, and each will be able to produce biologically useful sequence data very quickly. With the map, sequence data immediately slot into a context, rapidly expanding to large sequenced sections whose genetic information can then be analysed. Without the map, sequence data often remain enigmatic, isolated in fragments until much of the genome is completed. The researchers plan to sequence both DNA strands, as a check on the fidelity of the data. (Source: New Scientist, 25 August 1990)

D. APPLICATIONS

Pharmaceutical and medical applications

Mab diagnostic for myocardial infarction

Yamasa Shoyu (Choshi, Japan) has developed a monoclonal antibody that acts as a diagnostic agent for early identification of myocardial infarction, in joint research with Tokyo University, School of Medicine Professor Yazaki. The agent, Myosin II kit Yamasa, combines specifically with myosin, an insoluble protein that enters the circulatory system when myocardial cells die and remains present for about seven days. Yamasa has received approval from

the Ministry of Health and Welfare for production. Nihon Medifix will market the product to Japanese medical groups. (Extracted from Bio/Technology, May 1990)

Foetal test breakthrough

Researchers at Flinders University in Adelaide, Australia, have developed a test that will enable doctors to test for foetal abnormality as early as six weeks after conception. The test is non-invasive, and relies on assessing foetal cells present in a sample of the mother's blood. It has the potential to replace the current methods, amniocentesis and chorionic villus sampling, which are not usable until 10 to 16 weeks and which, being invasive, present small but not negligible risks. The technology, to be marketed by the University's commercial arm Flinders Technologies, has an estimated world-wide market of $250 million per annum.

The test, developed by a team led by Professor Warren Jones, relies on magnetic beads to which antibodies are attached locking onto the foetal cells; these can then be magnetically separated from the blood serum and analysed for genetic defects. The simplicity of the test will allow a much wider spectrum of pregnancies to be tested. It is expected that the technology could be available as a commercial product within two years. (Source: Australian Journal of Biotechnology, Vol. 4, No. 3, July 1990)

Gene triumph

Foreign genes have been safely inserted into the human body for the first time, according to a paper in the New England Journal of Medicine (vol. 323, p. 570).

Steven Rosenberg at the National Cancer Institute in Bethesda, and his colleagues, introduced foreign genes into the genetic material of white blood cells from five patients, using a weakened retrovirus as the carrier. They then returned the cells to the body.

The gene in this first experiment was not a therapeutic one, but a harmless bacterial gene coding for resistance to neomycin, an antibiotic.

The team was able to track altered cells in the blood of all five of the cancer patients three weeks after they received them. Two patients still had cells present in their blood two months later. No patient had any ill-effects or any trace of live retrovirus. (Source: New Scientist, 8 September 1990)

Some success with autologous gene therapy

Following the successful completion of the first authorized gene transfer study in humans, the Clinical Research Committee of the National Cancer Institute (NCI, Bethesda, MD) has approved a new study. It will use genetically modified tumour-infiltrating lymphocytes over-expressing tumour necrosis factor (TNF) to treat a small group of patients with advanced malignant melanoma.

The proposed study is the continuation of the work of Steven Rosenberg and his colleagues at NCI, which started in 1985 with the use of lymphokine activated killer (LAK) cells in adoptive immunotherapy against cancer. LAK therapy itself had been successful but controversial because of the severity of the side-effects. Rosenberg's group had first tried to identify cells that would have a more potent anti-cancer activity than LAK cells. This approach led to the identification of tumour infiltrating lymphocytes (TILs). Those isolated from a given patient's tumour (sperifically, in malignant melanoma) recognize tumour antigens that are present on cells of an autologous melanoma but not on normal cells from the same patient or on other tumour cells. TILs can recirculate through the peripheral blood system and can specifically localize to cancer deposits. When used to treat advanced metastic melanoma, 40 per cent of patients showed objective regression.

This targeting ability potentially makes TILs good delivery vehicles for molecules that could further increase anti-tumour activity. At the planning stage are projects in which recombinant TILs expressing genes for TNF, interferon or interleukin-2 (IL-2) are used in autologous transfer protocols.

In January 1989 Rosenberg's group obtained final approval for a seven-patient study in which TILs carrying the neo^R marker gene were used in patients with advanced malignant melanoma. The neo^R gene was inserted by retroviral transfer into autologous TILs and, after extensive safety testing for virus replication competence and IL-2 dependence, around 3×10^{11} cells were returned to the patient.

Biopsies showed that by three days after transfer of the cells, TILs were already present in the tumour nodules: after 19 days, the tumour was overrun with TILs. In one patient who is still disease-free 10 months later, the treatment resulted in the complete disappearance of 30 melanoma metastases.

The next step for the NCI group is to use TILs expressing TNF genes to treat other melanoma patients. In the run-up to the clinical studies, they have been able to insert the TNF gene into TILs and achieve a 20- to 90-fold increase in TNF production. In this way, they hope to achieve high concentrations of TNF within the tumour without producing toxic levels systemically. (Source: Bio/Technology, Vol. 8, August 1990)

Drug molecules "fused"

Immunex Corporation says it has genetically engineered a fusion molecule composed of two drugs - macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL-3) - that has 10 times the ability to promote bone marrow cell growth than combinations of the two.

The fusion molecule of the two therapeutic cytokines, called PIXY-321, could be the first of a second generation of CSFs, if proven safe and effective.

Previous animal studies showed that sequential administration of GM-CSF and IL-3 can lead to synergy in raising both white blood cell and platelet count. In vitro studies with the new fusion molecule indicate even greater synergy than has been seen in merely combining the two.

GM-CSF and IL-3, both compounds Immunex produces in yeast, are currently being tested to alleviate blood-cell damaging side-effects cancer patients suffer as a result of radiation or chemotherapy. Clinical studies of PIXY-321 are planned for 1991.

GM-CSF promotes the growth and function of infection-fighting white blood cells, while IL-3 stimulates early blood-cell development, including the production of platelets, the blood elements responsible for clotting.

Animals that received PIXY-321 responded with rapid increases in both platelets and white blood cells, an optimistic result, as it points to the combination of the benefits of different CSFs in a single drug.

Immunex says in vitro studies show PIXY-321 binds to receptors with higher affinity than either IL-3 or GM-CSF. This may be due to its ability to bind to three types of CSF receptors. the GM-CSF receptor, the IL-3 receptor and the "dual receptor", a unique receptor discovered by Immunex that is capable of binding GM-CSF or IL-3.

GM-CSF and IL-3 are currently in clinical studies in the US and Europe. Phase III clinical studies to reverse neutropenia, a deficiency of mature white blood cells caused by radiation or chemotherapy, are continuing.

Phase I/II clinical studies of IL-3 to test its effect on neutropenia and platelet deficiencies are also being conducted. Combination studies of GM-CSF and IL-3 are under way in Europe and are planned to begin in the US. (Source: Chemical Marketing Reporter, 3 September 1990)

Trials of growth factors show promise in cancer

Growth factors, proteins produced naturally by cells in the body, may help cancer patients suffering from the severe side-effects of treatments such as chemotherapy.

Growth factors regulate activity and division in cells, including cells of the immune system. They can even "boost" the immune system. Some can also slow down and suppress the activity of cells - a function that is arousing interest for future cancer therapies.

Scientists now have evidence that the purified proteins can help several groups of people, including patients suffering the effects of chemotherapy and those having bone marrow transplants for severe leukaemia. The clearest benefits so far seem to be in chemotherapy.

Two substances in particular - granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) - have produced promising results. The cell-killing drugs used to treat tumours kill healthy cells as well as malignant ones, and doctors often have to stop a course of treatment because an individual's supply of the white blood cells called neutrophils has fallen to dangerously low levels. Often, such individuals develop life-threatening infections as a result.

Both G-CSF and GM-CSF independently appear to stimulate the production of neutrophils in the bone marrow of these patients. The result in the majority of patients treated with these growth factors has been fewer infections after chemotherapy and shorter stays in hospital, according to Erich Platzer, from the University of Erlangen, Germany.

Several clinical trials involving more than 1,000 patients have now taken place in the US, Australia and Europe. Doctors give the growth factor as a daily injection. "The results have remained much the same across countries, institutions and continents", says Roland Mertelsmann, a leading researcher in growth factors from the Albert-Ludwigs University Medical Centre in Freiburg, Germany.

For example, a trial in Australia at the Ludwig Institute of the Royal Melbourne Hospital found that G-CSF reduced the period when patients' neutrophils were depleted to three to four days, compared with six days for controls.

However, some researchers have expressed fears that growth factors could end up stimulating tumour cells, as well as the body's defences. In the laboratory, high doses of growth factors have indeed stimulated overproduction in cultured leukaemic cells. But, according to Mertelsmann: "There is zero evidence in vivo that G-CSF will stimulate tumours." No patient would ever receive doses as large as those tested in the laboratory, he said. (Source: New Scientist, 1 September 1990)

New drug is tough on tumours

A drug to stop cancer tumours spreading is to undergo clinical trials in Manchester early in 1991. The drug will be tested in breast cancer patients with secondary bone tumours in the world's first trial for a drug of this type.

Tumour cells secrete the enzyme collagenase which breaks down collagen in the tissues surrounding it, allowing the tumour to spread around the body. A collagenase inhibitor occurs naturally in the body to control the effect of collagenase and the new drug has been designed to mimic this inhibitor.

British Bio-technology researchers discovered a specific reaction site on collagenase responsible for its activity. They then designed a drug that would bind to this site and inhibit the action of collagenase.

Since the collagenase inhibitor does not kill cancer cells it will have to be used with anti-cancer drugs. However, it is often the spread of secondary tumours which is the most difficult to treat.

Since the drug is not cytotoxic it is likely to have few side-effects. The animal data supporting the proposed trial are "very convincing".

British Bio-technology is already collaborating with SmithKline Beecham on anti-arthritis drugs that work on the same principle. These drugs are designed to prevent the breakdown of tissues in the joints of arthritis sufferers. (Source: Chemistry and Industry, 17 September 1990)

Nuclear matrix proteins yield first monoclonal breast-tumour marker

A monoclonal-antibody kit for finding and tracking breast cancer is on the market - but for research use only. Matritech, Inc. of Cambridge, Mass. describes its first product as a monoclonal that "detects a novel nuclear matrix protein (NMP) found in high concentrations in breast carcinomas".

Most marker proteins in current use for detecting malignancy are based on so-called "tumour-associated antigens", such as CEA (carcino-embryonic antigen) and AFP (alpha-foetoprotein). Both of these cell-surface proteins occur normally during foetal development, and recur

abnormally in some malignant tumours. But like oncogenes, which are also tumour-related, tumour-associated antigens "can be present even though there is no malignancy, and absent in the presence of a malignancy", points out Douglas C. Pearl, Matritech's director of product management.

Matritech is pinning its hopes, and its R&D programme, on the discovery that "certain purified nuclear matrix proteins have been shown to be cell-type specific; others are differentially expressed by normal and malignant tissue", explains Pearl.

The firm expects that its patented technology will permit diagnosing three properties of cells in a tumour biopsy or blood sample: tumour type, tissue of origin and degree of malignancy. (Extracted from McGraw-Hill's Biotechnology Newswatch, 16 July 1990)

Bacterial treatment for bladder cancer

Connaught Laboratories has received US Food and Drug Administration approval for its live bacterial treatment for mild bladder cancer. The technology involves flooding the bladder for two hours once a week for six weeks with the bacterium used in tuberculosis vaccines. The solution of Bacillus Calmette-Guerin produces an inflammation that destroys the cancer cells. The treatment is then performed monthly for six to 12 months. About 20 to 30 per cent of the 45,000 cases diagnosed each year might be treated with the new technology. More severe cases will still be treated with surgery and chemotherapy. Tests have shown the new treatment to produce results in 74 per cent of suitable cases, compared to a 42 per cent response rate with the cancer drug adriamycin. Spread of the bacteria from the bladder can be a fatal side-effect of the treatment. (Extracted from New York Times News, 22 May 1990)

Synthetic molecule may stop spread of HIV

The latest candidate in the search for an AIDS drug is based on a small, synthetic molecule whose off-the-shelf components take only four steps to assemble. A collaborative project by chemists and biologists in the US has already begun trials of the compound, CPF(DD) in mice. Within a year the team will know whether they can start to test it in humans.

If it proves a safe and effective therapy, CPF(DD) could be used alone or with zidovudine, the only licensed AIDS drug, say its inventors. But they stress that the research is still at an early stage.

Steven Burakoff, Stuart Schreiber, Robert Finberg and their colleagues at Harvard University have refined an existing strategy, based on established knowledge of the virus, for preventing HIV from binding to human cells.

A protein on the virus's coat, gp120, latches onto a receptor molecule on the cell surface known as CD4. Researchers have reasoned for several years that a genetically engineered form of CD4 could "mop up" HIV in the blood, blocking gp120 and stopping the virus from binding to the genuine CD4 molecule on the cell. However, CD4 is a big molecule, and the synthetic form may be broken down by enzymes in the body before it can do the job. Its performance in stopping HIV from binding to cells has so far disappointed many AIDS researchers.

The Harvard team decided to work out exactly which part of CD4 was crucial and design a small molecule that might survive longer in the body. By analysing mutated forms of the protein they found that one amino acid, phenylalanine, was essential.

Next, they modelled various small molecules bearing phenylalanine, calling them CPFs (for N-carbomethoxycarbonyl-prolyl-phenylalanyl benzyl esters). In the test tube, CPFs stopped gp120 from binding to CD4 on cultured human cells. They also stopped the virus from spreading to uninfected cells.

CPFs have only two amino acids, compared with 400 in CD4. In addition, they contain a dicarbonyl group and a benzyl ester.

One CPF in particular worked well. CPF(DD) has its amino acids arranged in the mirror image of their natural counterparts, so the body takes longer to break the molecule down.

The next vital step is to find out whether the molecule is safe to use. With this in mind, Burakoff and his colleagues have given it to mice in very high doses - at least 20 times as much as necessary. The preliminary results of this trial - measured in terms of the survival of the mice - suggest CPF(DD) is not toxic to the animals.

Within two months the team hopes to start trials of the molecule in a special strain of mice that lack their own immune system - so-called SCID mice. These animals, developed by the Medical Biology Institute at La Jolla, California, have been injected with cells of the human immune system. (Source: New Scientist, 28 July 1990)

Trials planned for new AIDS vaccine

The Oxford, UK-based biotechnology company British Bio-technology (BB) will start clinical trials of a potential AIDS vaccine in the UK in September. Phase I trials in healthy human volunteers will be held at the Hammersmith Hospital in London, in conjunction with the Medical Research Council's (MRC) AIDS programme.

The vaccine is based on BB's proprietary technology for producing immunogenic "virus-like particles" (VLPs). These are grown in yeast cells and resemble viruses in shape and size but do not replicate. The technique was originally developed by BB over three years, centering on work done at Oxford University.

The vaccine, known as p24-VLP, expresses the p24 protein, a non-mutating core protein of the HIV. The vaccine's active agent was produced by isolating the gene which expresses the p24 and introducing it into the DNA of the VLP-producing yeast.

The initial trials will measure the quality and duration of immune response. The MRC has, however, stressed that procedures are in place so that no volunteers in the trial are mistakenly thought to have HIV infection. (Source: European Chemical News, 30 July 1990)

Clinical trials in rheumatoid arthritis patients

Synergen Inc. announced that testing of its anti-inflammatory agent, Interleukin-1 receptor antagonist (IL-1ra), began in patients with rheumatoid arthritis.

The initial clinical trials will focus on safety and appropriate dosing levels. Subsequent

trials at medical centres across the United States will study IL-1ra's effectiveness in reducing the pain and joint destruction associated with advanced rheumatoid arthritis.

In laboratory tests, administration of IL-1ra in animal models of rheumatoid arthritis has significantly and safely reduced the swelling and cartilage destruction associated with the disease. Synergen's scientists believe that these positive results stem from IL-1ra's ability to counteract the detrimental action of Interleukin-1 (IL-1), a primary mechanism for inducing inflammation. Elevated levels of IL-1 are found in patients with several inflammatory diseases, including rheumatoid arthritis. Synergen's scientists have demonstrated that IL-1ra prevents IL-1 from inducing inflammation in these pre-clinical models by competing for the same receptors on cellular surfaces.

Current therapies for rheumatoid arthritis include pain relievers and non-steroidal anti-inflammatory drugs, which provide symptomatic relief, but do not slow or stop the disease's progress.

Synergen also announced that its anti-inflammatory agent, Interleukin-1 receptor antagonist (IL-1ra), significantly reduced tissue damage and inflammatory cell infiltration in an animal model of ulcerative colitis.

Ulcerative colitis and Crohn's disease are forms of inflammatory bowel disease (IBD). Patients suffering from IBD frequently require extended hospitalization. Assuming continued favourable results, Synerger expects to begin clinical trials in 1991 to test IL-1ra as a treatment for IBD. (Source: Company News Releases, 7 and 13 September 1990)

Seatec of USSR to sell digestase

Dr. Michael Trevan, head of the Department of Biotechnology at London's South Bank Polytechnic, has completed an agreement with the Seatec Co. in Moscow under which South Bank Polytechnic Enterprises Ltd. will market a new enzyme - Digestase - internationally. Seatec have produced the enzyme from the organs of crabs farmed in the Kamachtka peninsula region of the Soviet Union.

At present, enzymes used for the tenderization of meat are purified from various plants and micro-organisms. They break down the meat structure, making it more easily digestible. However, these enzymes tend to break down the meat rather than the indigestible elements - and are expensive. The new enzyme is extracted by purely physical means from crab organs that would otherwise be discarded. Patented in the USSR, Digestase is expected to be in considerable demand.

Given the enzyme's ability to break down the tissues connecting cells, it can also be used at biopsy stage to break down cancerous tumours into individual cells. From these individual cells, antibody molecules can be formulated. These can then be put back into the body, where they fight other cancerous tumours. Details from: Dr. Mike Trevan, Department of Biotechnology, South Bank Polytechnic, 103 Borough Road, London SE1 0AA. (Source: Biotechnology Bulletin, Vol. 9, No. 7, August 1990)

Hepatitis drug success

US scientists have reported that Schering-Plough's genetically engineered interferon alpha-2b Intron A is a successful treatment for chronic viral hepatitis, which by coincidence followed an FDA recommendation that the same drug be approved to treat hepatitis C in the US.

The scientists reported results from a multi-centre placebo controlled trial of Schering-Plough's version of interferon. A sustained loss of viral replication was reported in over a third of patients suffering chronic hepatitis B. Moreover, in about 10 per cent of patients on interferon, hepatitis B surface antigen disappeared from serum.

World-wide, the incidence of hepatitis is rising, according to Robert Spiegal, Schering-Plough's vice-president of research. In Japan it has assumed "epidemic" proportions. Sales of the drug surged to $92 million for the first half of this year, compared with $92 million for the whole of 1989.

Schering-Plough's interferon alpha-2b, Intron A, is one of three versions of genetically engineered interferon alpha on the US markets. Hoffmann-La Roche markets Roferon A and Wellcome sells Wellferon. Current indications are a range of rare cancers, such as hairy cell leukaemia and Kaposi's sarcoma. (Source: European Chemical News, 13 August 1990)

Cetus IL-2 therapy suffers double set-back

The Californian biotechnology firm, Cetus, has suffered two blows to the development of its genetically engineered interleukin-2, Proleukin. The FDA's decision not to recommend it for the treatment of metastatic renal cell carcinoma (kidney cancer) followed soon after clinical work on the drug's antihypertensive effects was suspended, pending validation of the pre-clinical results.

Clinical trials have shown that IL-2 produces significant and lasting responses, providing benefit to about 20 per cent of kidney cancer patients. In common with most anti-cancer agents, IL-2 has many side-effects but these are predictable and manageable and reverse once the therapy is concluded.

The enrolment of new patients in the phase I clinical development of Proleukin for treating hypertension was suspended because independent scientists have been unable to repeat the original pre-clinical results upon which the trials were based.

In January, researchers at the Masonic Medical Research Laboratory, in Utica, New York, reported encouraging results for IL-2 as a potential hypertension treatment in a rat model. Based on this, human clinical trials were started. However, these have not progressed sufficiently to provide any information on efficacy. According to the company, the additional pre-clinical work is likely to take several months. (Source: European Chemical News, 13 August 1990)

Firms report AIDS vaccine progress

First results for human clinical trials of a potential AIDS vaccine developed by the Biocine company, a joint venture between Ciba-Geigy Corp. in the US and Chiron, have shown it to elicit an HIV-specific immune response.

Experiments based on four healthy male volunteers conducted at Geneva University Hospital showed the production of HIV specific T-helper cells. These cells recognize the surface protein of three different HIV variants and respond specifically with cell division, a type of

reactivity known in immunology as cross-reactivity, indicative of broad HIV specificity.

The experimental vaccine consists of an HIV isolate, genetically engineered from yeast, combined with an adjuvant, or immune response enhancer, developed by Ciba-Geigy in Basle.

Ciba-Geigy said the results would enable an improved vaccine to be formulated which would enter trials in the US later in the year.

Other scientists have made progress on a potential AIDS vaccine. Researchers from the US biotechnology company Repligen, which is co-developing a potential vaccine with Merck & Co., say that the variability of the HIV does not make the development of a broadly effective AIDS vaccine unfeasible. The findings, based on an analysis of the key variable HIV fragment, RP135, demonstrate that a small number of virus families exist, each with a similar RP135 structure. A cocktail of between five and 10 RP135 structures may provide protection against most of the known strains of HIV in North America and Europe.

The two companies are developing an appropriate delivery system for their RP135-based vaccine, which would boost immune response and extend the effective life in the body. The next step is for toxicity and efficacy tests in animals to be conducted. (Source: European Chemical News, 3 September 1990)

Drug success in AIDS study

Early findings indicate that Bristol-Myers Squibb Company's experimental drug DDI may prolong AIDS patients' lives as long or longer than AZT. US Government scientists say their study of 58 patients with AIDS or a complex of AIDS-related symptoms, called ARC, show 88 per cent of patients were alive 21 months after starting DDI, or dideoxyinosine treatment.

That compares with a 50 per cent survival rate at 21 months among similar patients in early tests of AZT, or zidovudine, and the 25 per cent survival rate at 21 months found in the past among patients who received no treatment.

Dr. Robert Yarochoan of the National Cancer Institute emphasizes that the study sample was small and did not directly compare the effectiveness of AZT, which is made by Burroughs Wellcome Company, with DDI. Tests to directly compare the two drugs in up to 2,500 patients are now under way, and results should be available late next year.

Neither AZT nor DDI, which both attack an enzyme the virus needs to reproduce, is a cure for AIDS. However, both appear to slow the disease's lethal progression.

Because the two AIDS drugs carry different toxicities and likely also generate different drug resistance, researchers hope it may be possible to extend AIDS patients' lives even further with treatments that alternate the two agents. (Extracted from Chemical Marketing Reporter, 3 September 1990)

Pokeweed anti-HIV?

US scientists are developing a novel anti-AIDS compound based on a protein with antiviral properties isolated from the pokeweed plant.

Joyce Zarling and colleagues at Oncogen in Seattle have described how the pokeweed antiviral combined with a monoclonal antibody (Mab) is able to target immune cells infected by HIV-1 and inhibit replication of the virus at picomolar concentrations. The scientists believe that at this low concentration the pokeweed-derived protein has little effect on normal immune responses.

The Mab is targeted to recognize CD4 receptors on immune cells. These are the principal targets to which the HIV binds in order to enter and infect the cells. (Source: European Chemical News, 10 September 1990)

AIDS trial expands

Australia, Switzerland and the Netherlands have now joined the Anglo-French trial of the experimental AIDS drug dideoxyinosine (DDI). More than 100 British and 250 French people with AIDS have enrolled since the start of the trial, which is organized jointly by the Medical Research Council in the UK and its French counterpart, INSERM.

DDI causes inflammation of the pancreas - which has occasionally been fatal - in about 1 per cent of users. Doctors accept that fears of pancreatitis may have deterred some individuals from joining the trial. However, researchers in the US and the UK say they are now identifying the factors that put patients at risk from the complication. Those most at risk are people who have already suffered from pancreatitis. (Source: New Scientist, 8 September 1990)

Trial approved for saliva test

A novel saliva-based test for HIV (human immunodeficiency virus) infection, which may ultimately lead to a "do-it-yourself" AIDS test, has been approved by the US Food and Drug Administration (FDA) for human clinical trials. The technique, developed by Epitope Inc. of Beaverton, Oregon, may make AIDS testing simpler and more widely available because antibodies are collected from the mouth without the need to draw a blood sample.

In the test, a treated pad is placed between the gums and cheek for two minutes to collect antibodies to HIV, which are present in the saliva. The pad is then placed in a vial and mailed to a laboratory where it is submitted to the standard HIV test now used on blood samples. In pre-clinical trials on 600 patients, Epitope scientists report that the collection device has been perfectly reliable, leading them to believe the test will be "fully equivalent" to blood tests.

FDA approval will allow Epitope to carry out clinical trials for nine months at five sites nation-wide, where matched samples of blood and saliva will be compared. If marketing approval is then granted, the company plans to make the device available world-wide. Potential users include police and emergency medical personnel who request frequent testing for HIV, and developing countries where blood testing may be impractical. (Source: Nature, Vol. 346, 23 August 1990)

Antibiotic substance found to be active against HIV

Scientists at Daichi Seiyaku and Tokyo Medical and Dental University have discovered that a synthetic antibiotic denoted DR3355, currently undergoing clinical trials, appears to be active against HIV. According to Japan High Tech Report, the joint research group found that when added in vitro to HIV-infected human lymphotyces, DR3355

led to the complete disappearance of the virus within 30 days. (Source: European Chemical News, 24 September 1990)

Livestock applications

Rabies vaccine

The first field tests of a genetically engineered rabies vaccine are under way in the United States - some four years after field tests were conducted in Canada and months after a full-scale campaign to eradicate rabies from the fox population began in France.

Rabies is now at epidemic proportions in the raccoon population in the mid-Atlantic states and there are fears it could spread to domestic animals, including pet cats and dogs. The vaccine is a recombinant vaccinia-virus vaccine and is the product of collaborative research between the Wistar Institute of Philadelphia and Transgene of Strasbourg, France.

Trials are being carried out on the uninhabited Parramore Island off the Virginia coast. The vaccine is placed in a bait containing fish oils that are abhorrent to man and other animals but attractive to raccoons. Blood tests will be carried out for up to a year on raccoons to determine the level of protection against rabies. (Source: Nature, Vol. 346, 30 August 1990)

Anti-reproductive vaccine

Australian biotechnology claimed a significant achievement in the last few days of June when Arthur Websters and Peptide Technology jointly launched "Vaxstrate", the world's first commercial anti-reproductive vaccine. The product has been developed after several years' collaboration between the two industrial partners and the CSIRO Division of Animal Production and Tropical Animal Production.

The product is also believed to be the first synthetic peptide-based vaccine ever to be commercialized. Whilst applications in other animal species are being examined, the vaccine is designed primarily to play a role in Australia's beef industry: it prevents unwanted pregnancy in cows in the extensive grazing country in the far north. Pregnancy at slaughter greatly downgrades the value of the carcass, yet is a highly probable event in the vast northern tracts where cattle roam free for the whole year. The vaccine is claimed to be about 80 per cent effective under these husbandry conditions, and thus provides a very useful management tool where other preventative measures have been crude or impractical. (Source: Australian Journal of Biotechnology, Vol. 4, No. 3, July 1990)

New vector combats Newcastle disease

Epidemics of Newcastle disease continue to threaten the poultry industry, both in countries that control the disease by slaughtering infected flocks and in those that rely on the present generation of vaccines, which do not always afford solid protection. Now, a vaccine that uses fowl-pox virus (FPV) as a vector to carry genes coding for Newcastle disease virus (NDV) antigens can completely protect chickens against a virulent strain of the disease.

The new vaccine has been developed by Michael Boursnell and colleagues at the Houghton Laboratory of the Agricultural and Food Research Council's Institute for Animal Health. The British Technology Group (BTG, London) has financed their work in part. BTG has patented gene constructs coding for antigenic determinants of NDV and infectious bronchitis virus in recombinant viruses developed at Houghton.

The Houghton group has carried out a considerable amount of work on the organization of the FPV genome, including the identification of its topo-isomerase gene. Sequencing has shown that this is 56 per cent homologous with its vaccinia homologue. Boursnell and co-workers have also located, in the FD8 and FD9 genes within the central region of the FPV genome, non-essential sites into which foreign genes can be placed. They believe that these will be useful in further work on the construction of polyvalent vaccines. The genetic and environmental stability of FPV, and the simplicity with which it can be cultured, also commend this organism as a safe vehicle to express protective antigens in non-avian species. (Extracted from Bio/Technology, Vol. 8, July 1990)

Agricultural applications

Corn bullets

Ciba-Geigy Agrochemicals has announced a new genetic-engineering method that could immunize commercial varieties of corn against attack from disease and pests for generations.

Scientists at Ciba-Geigy's Agricultural Biotechnology Research Unit in Raleigh, North Carolina, US, call the new method Biolistics because it involves shooting minute metal particles coated with genetic molecules into plant cells at high velocity.

The researchers say that Biolistics can give genetic resistance to cereal crops, such as maize and wheat, that have been less responsive to established techniques. Ciba says it will take eight years for engineered cereals to reach the market. (Source: European Chemical News, 3 September 1990)

Bio-insecticide gets US go-ahead

A bio-insecticide developed by Repligen Sandoz Research from a natural soil micro-organism has been approved by the US Environmental Protection Agency for use in field tests.

The bio-insecticide is based on an engineered strain of the micro-organism Bacillus thuringiensis (Bt). The new strain is expected to prove more effective than current sub-species in preventing crop damage caused by caterpillars, says the Repligen/Sandoz joint venture.

In laboratory tests the effectiveness of the modified strain was between two to three times greater than that of current Bt strains, claims the company. Field trials will be conducted within the next few months. A commercial product could be available in three to four years. (Source: European Chemical News, 1 October 1990)

Potatoes and peas make a meal of pests

Genetic engineers have produced a crop of potatoes modified to resist pests. This is the closest the UK has come to a full-scale agricultural harvest of genetically altered produce. Until now most field trials of engineered crops have been

grown from tissue culture taken straight from a laboratory, but this crop, planted in Norfolk by a seed company called Nickerson International, was grown from tubers, so is more representative of a conventional crop.

The Desiree potatoes contain a gene transferred from peas, known as the pea lectin gene, which should protect the plants from insect pests. The lectin protein interferes with the digestive processes of insects, preventing them from absorbing other vital proteins. This should stop an epidemic of pests such as the Colorado beetle or tuber moth.

The foreign gene is incorporated into the plants using Agrobacterium tumefaciens. The barterium is normally responsible for forming tumours, but can be altered so that it will act only as a carrier, inserting the foreign gene into the genetic material of the potato plant.

This latest trial builds on research into the pea lectin gene at the University of Durham. The company now hopes to gain approval for field trials of the modified potato plants next year in Israel and in the US, where potatoes are currently sprayed with chemicals to keep down the pest population.

Nickerson says its work could also lead to plants protected from pests that are more common in the UK, such as the nematode worms that attack root systems. These pests are particularly difficult to treat with traditional pesticides.

The field trial in Norfolk was approved by the Advisory Committee on Release to the Environment (ACRE), the body set up to monitor the safety of such tests outside the laboratory in the UK. (Extracted from New Scientist, 8 September 1990)

Recombinant rice flies from UK, dives into Arizona field tests

Transgenic rice seedlings hand-carried from Nottingham, UK, were planted in Maricopa, Arizona in a specially designed 10-metre long swimming pool. The rice - flown from a country too cold to sustain it to a state too dry - will teach researchers about field-trial methodology as well as plant physiology, says plant pathologist Wolfgang Schuh, Pennsylvania State University, University Park.

Arizona was chosen as the test location precisely because there is no rice grown there. In a state where rice is grown, the heads would have had to be bagged (to avoid cross-pollination), which would alter micro-climatic conditions. If it was extremely hot, it might change the data. Seed viability might be different. Schuh adds that the absence of other rice will allow researchers to track seed migration, determining the degree of isolation necessary for future rice field experiments.

"This is a very basic trial", states botanist Edward C. Cocking, School of Biological Sciences, Nottingham, UK. "We want to find out what happens to agronomic characteristics when we introduce a foreign gene." His rice carries a kanamycin-resistance gene.

Cocking is also trying to develop hardier domestic rice through introduction of genes from wild strains, nitrogen fixation and resistance to tungrow virus. Cocking does not plan to patent any of the technology. "There is little commercial interest in rice", he explains. "Profit cannot be made readily from a half-million poor farmers. We publish as quickly as possible, to ensure that the methods can be readily transferred to developing countries." Cocking expects to see recombinant rice reach farmers in "three to five years". (Extracted from McGraw Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

A green solution to the green revolution

The success of the Green Revolution depended on farmers switching to high-yielding varieties of crops and intensive use of fertilizers and pesticides. Not only is such technology beyond the reach of poorer farmers, but the intensive use of agrochemicals in the third world is leading to environmental and health problems.

One small peasant farmer on the island of Panay in the Philippines has developed an integrated system of farming that increases the yields of his land more than any Green Revolution, and yet requires no chemical input. The originator is 63-year-old Mamerto Fantilanan, and his system is based on azolla and the principle of "giving back to nature what you take from it". Fantilanan's half-hectare irrigated farm provides total self-sufficiency for a family of 13, in addition to producing a net annual income of 70,000 pesos (about $3,000), more than twice the income of nearby farmers with four times the amount of land.

Azolla is a small aquatic fern which can double its mass every three to five days. It also fixes nitrogen from the atmosphere through a symbiotic association with the blue-green alga, Anabaena azollae. Azolla can be used both as a fertilizer and as an animal feed, but few farmers have recognized its full potential.

Fantilanan grows two species, Azolla pinnata and A. microphylla. Some of the crop he uses to feed his pigs, ducks and chickens. The effluent from the piggery is mixed with five parts of azolla and fed into a biogas digester. This provides methane fuel for heating and lighting the farmhouse and for warming incubators in a small hatchery. He uses the residue from the digester, which is odourless, as fertilizer for the rice and a wide variety of other vegetable and fruit crops, including bananas, guava, papayer, star fruit and coconuts.

Azolla can even be consumed by humans - it contains between 22 and 37 per cent protein - either directly or as azolla omelettes and azolla burgers. Apart from this versatility, its rapid growth and ability to fix nitrogen (each crop produces equivalent to applying 30 kilograms of nitrogen per hectare), the continuous mat of azolla which forms in rice paddies acts as a weed suppressant. Experiments at the International Rice Research Institute, near Manila, showed that it can reduce the total weed mass by 72 per cent. Despite the obvious potential of azolla, scientists at the IRRI estimate that the fern is probably used on less than 2 per cent of the world' total rice area of 150 million hectares.

The secret of Fantilanan's success is not just azolla, but the integrated system of farming he has developed. For example, the farm contains a series of ponds. One holds Tilapia nilotica which he feeds exclusively with azolla. Ducks occupy another pond, situated next to a golden snail pond and a clam pond which produces clams weighing up to 500 grams. The family sells these, along with surplus fish, snails and vegetables, to restaurants as a lucrative sideline. The snail pond is kept stocked by snails

removed from the adjacent rice paddies, where they are a major pest.

Fantilinan's integrated system, although involving simple technology, represents a highly scientific approach to farming. It incorporates the combined wisdom of generations of Asian farmers with modern developments.

Despite the overwhelming evidence of the success of this integrated organic method, a number of major obstacles to its replication exist. Although successful growth of azolla depends on a plentiful supply of phosphorus and abundant water at all times, Fantilanan's Green Solution requires virtually no artificial inputs. As no inputs means no profits for the agrochemical multinationals, it is likely that they will resist the widespread growth of the technique. (Extracted from New Scientist, 25 August 1990)

Weeding with fungi

Mycoherbicides, fungi that kill weeds, promise to deliver what other herbicides cannot: a highly specific treatment aimed at a single species of weed that will leave other plants untouched. Pathologists and ecologists now recognize that many wild plants are afflicted by some extremely damaging diseases. As in crops, the symptoms of disease - spotty or yellowing leaves and distorted stems, for instance - may be similar on different plants, but such similarities hide the fact that many different fungi cause these infections and that many of them are highly specific to their hosts. Each plant has its own collection of pathogens, some of which are unique to it. The realization that certain fungi do immense damage to weeds while leaving crops untouched has led to a change of perspective in plant pathology.

Scientists in the UK and Europe are now following the example of American researchers in developing mycoherbicides from fungi that are natural pathogens of the target weed.

The plants chosen for this "specificity screen" are not picked at random. A pathogen that infects a particular plant is most likely to attack other species that are closely related to it. The specificity screen is designed to include many species from the family to which the weed belongs and a few species from less closely related groups.

Specificity alone is not enough to make a good mycoherbicide. The fungus must satisfy several other criteria. It must grow and produce spores readily in cultures in industrial conditions, preferably in a liquid medium in a standard industrial fermenter. This ability is not as common as manufacturers might wish, even among fungi that readily form spores in laboratory cultures. Once the identity of the fungus is confirmed, patents must be available to the developer - which means that no one else must have exploited that particular fungus before. It is also important that other pesticides (fungicides, insecticides or herbicides) applied to the crop do not harm the fungus.

All these factors are vitally important, but there is yet another hurdle to leap before a fungus is ready for development as a weedkiller. All the earlier tests involved parts of plants or young seedlings. In the field, the mycoherbicide's target would be older, much hardier plants. The best way to ensure that the fungus has a chance to work on these tougher individuals is to design a suitable formulation in which to apply it - one that lengthens the life of the spores, for instance, or which increases their stickiness so that they cling to the leaf long enough to germinate.

With so many criteria to meet, almost all natural isolates fail at one or other obstacle. Many fall at the first fence, for while they might infect the weed, they are not destructive enough to control it in the field. The relatively low virulence of most isolates is of no great surprise: a pathogen that eliminates its hosts is likely to die out itself unless it can survive in dead tissue, which most of these fungi cannot. What is more surprising perhaps is that very damaging isolates do exist, but they do not wipe out their hosts because they generally infect only pockets of the host population. This highlights the key role of environmental factors in limiting the natural spread of disease. The production of spores and their transmission between hosts are highly dependent on suitable weather conditions. These rarely allow severe natural epidemics to develop.

The usefulness of mycoherbicides depends on the ability to remove these constraints by inundating the weed with massive doses of spores formulated so that they are no longer dependent on a narrow range of environmental conditions. In the US, two mycoherbicides, Collego and Devine, have been on the market for almost 10 years. Both are targeted at weeds that are not very widespread: northern joint vetch, the target for Collego, infests rice and soya bean crops in Arkansas, Louisiana and Georgia, and milkweed vine, Devine's target, affects citrus groves in Florida.

The success of these two fungal herbicides has paved the way for new products aimed at more widespread weeds. One of these, Cassl (Alternaria cassiae), is aimed at two weeds: sicklepod (Cassia obtusifolia) and coffee senna (C. occidentalis) which damage soya bean and peanut crops throughout the southern US.

The European Weed Research Society has recently drawn up a "Top Ten" of European weeds that are suitable targets for mycoherbicides. The selection is based on the extent of the economic damage these weeds do throughout Europe and, in some cases, because of their resistance to chemical herbicides. The list includes such well known enemies as chickweed and cleavers. At Long Ashton Research Station near Bristol, Mike Greaves and his colleagues are studying two of the Top Ten, cleavers (Gallium aparine) and bindweed (Convolvulus arvensis). They have isolated a number of fungi that show promise in controlling these two weeds. Researchers at Shell's Research Laboratories in Sittingbourne, Kent, are investigating other weeds on the hit list.

Alan Watson, at McGill University in Quebec, Canada, has shown that while neither the mycoherbicide Colletotrichum coccodes nor the chemical herbicide thidiazuron totally and reliably controls velvetleaf (a weed of corn and soya beans), a combination of the two will bring the pest under control. If chemical and fungal weedkillers act in concert then mixtures of the two could be a way of reducing the doses of chemical herbicides and their residues that linger in the environment.

Such synergism also means that some of the weaker fungal pathogens might be suitable as weedkillers, if they are combined with a chemical. The fact that a fungus may remain pathogenic in the presence of a chemical herbicide also means that the fungus might be used alongside chemicals in circumstances where a whole spectrum of unrelated weeds needs controlling.

Fungi can be combined with other fungi and with insect controls as well as with chemicals. At Lancaster University, Steven Hallett has found that when certain fungi are applied in pairs, they achieve together a "kill" that is impossible with either fungus alone. Mycoherbicides are an additional option in programmes of integrated pest management, particularly in partnership with insects or other invertebrates. Water hyacinth is a good example of a pest that succumbs to this approach. Water hyacinth is a serious weed in watercourses, hindering navigation and eliminating native wildlife. Neither the fungus Cercospora rodmanii nor insect controls, such as Neochetina weevils, can curb the plant alone. But in combination the fungus and insects kill more than 99 per cent of the weed.

Mycoherbicides have several advantages over conventional chemical herbicides. The search for mycoherbicides is more directed, reducing the costs of development. This could make them an attractive commercial option. Mycoherbicides could also be used as alternatives to chemicals where the weed has developed resistance. At least 48 species of weed are now resistant to the once widely-used triazine herbicides. Mycoherbicides are also more selective than most chemicals, making them a better choice for controlling weeds that are close relatives of the crops that they infest, such as fat hen in crops of sugar beet (both belong to the family Chenopodiaceae).

Mycoherbicides clearly offer a way of reducing the amount of potentially toxic chemical herbicides entering the environment. Because the fungi are not animal pathogens they probably pose little risk to human and animal health. Nevertheless, before any mycoherbicide receives a licence, it is screened to rule out any hazards, such as allergic reactions among farmers. A more serious concern is that mycoherbicides might threaten natural vegetation. So far, however, the development of mycoherbicides has involved only fungi that occur naturally in the environment. An application of a fungus simply increases the size of the local population of the organism. Most importantly, unlike many chemical herbicides, mycoherbicides are not persistent. In the absence of its host weed, the fungal population soon diminishes.

Genetic manipulation of fungi might be on the cards in the future, but long experience with diseases of crops has shown that host-specific, native pathogens do not normally "jump" to new hosts. Even if native strains were improved, so that they secreted more of the enzymes or toxins that destroy host tissue, for example, they are likely to pose little risk. They will simply be producing more of what they already produce in nature. If fungi could be genetically engineered to control weeds that are not their normal host, the position would be rather different. What is certain is that increasing numbers of new mycoherbicides based on native pathogens will appear on the market in the next few years. What is also certain is that the first products must be beyond reproach. Any mistakes at this early stage could damage irreparably the growing reputation of this novel approach to weed control. (Source: New Scientist, 1 September 1990)

Food production and processing

New beer yeast developed

A genetically engineered yeast that can ferment diet beer has been developed at the Brewing Research Foundation's laboratories in Nutfield, Surrey. Low carbohydrate beers are currently made by adding the enzyme glucoamylase from the micro-organism Aspergillus niger to the fermentation. The enzyme then breaks down the dextrin molecules of beer into fermentable glucose.

Researchers at the Foundation have cloned the glucoamylase gene from Aspergillus niger and inserted it into the brewing yeast so that the yeast can produce the glucoamaylase itself. The high alcohol, low carbohydrate beer, known as Nutfield Lyte, tastes "very acceptable" and is currently being brewed in pilot fermenters at the laboratories. (Source: Chemistry & Industry, 17 September 1990)

Method could produce sweeter fruits and vegetables

A new genetic engineering method called Transwitch could lead to sweeter fruits and vegetables as well as plants with longer shelf life according to the developer, DNA Plant Technology. The technique enables plant breeders to "switch off" expression of specific genes, allowing scientists to alter the sweetness and shelf life of fruits and vegetables or the fatty acid composition of edible oils.

The Transwitch process begins with the identification and cloning of a specific gene in the plant cell. The duplicate gene is then reinserted in the chromosome. The "double gene" has a paradoxical effect. Instead of twice the expression of a particular characteristic, the genes frequently cancel each other, eliminating the trait. (Source: Food Engineering International, August 1990)

Pathological bacteria detection

Shimadzu (Kyoto, Japan) has developed a process for detecting pathological bacteria by DNA identification. The test is based on a reagent that attaches a fluorescent marker to a specific gene DNA sequence that can be used to identify the bacterial strain. The test takes five hours to perform, compared to more than two or three days with conventional culturing methods and can detect the presence of very low levels of pathological bacteria (roughly 100 bacteria in a sample). Markers have been developed for salmonella, Welch bacillus and six other bacteria associated with food poisoning. (Extracted from New Technology Japan, May 1990)

Pasteur's r-DNA test picks Listeria perpetrator out of genus line-up

A food-poisoning bacterium, Listeria monocytogenes, found mainly in unpasteurised cheese and undercooked sausage, should be easier to pinpoint in food products and patients, if and when DNA tests developed at France's Pasteur Institute reach the market. Molecular biologist Pascale Cossart of the Institute's Genetic Microbiology Unit reported that her unit's technique "is the only one available that specifically identifies Listeria monocytogenes" - the only virulent strain of Listeria. "Other techniques", she added, "identify only the entire genus Listeria - i.e. the one pathogenic and six non-pathogenic species".

Moreover, the Pasteur scientist stated, "... results are obtained in two days of cultural enrichment, compared to the six-to-eight-day FDA method ... the only technique available which specifically identifies the pathogenic species Listeria monocytogenes".

Pasteur's test for detecting the virulent strain of Listeria in foodstuffs, by PCR amplification of bacterial DNA, is already being offered to French dairy companies.

In France, listeriosis strikes an estimated 1,000 victims a year, mainly pregnant women and other immuno-compromised persons, killing 30 per cent of them. Another 5 to 10 per cent suffer serious sequellae- such as meningitis. When ingested by a mammal, a L. Monocytogenes bacterium is in turn ingested by phagocytes - key players in the body's waste-disposal system. The pathogen's virulence derives from a protein it secretes, listeriolysin O (LLO), which enables it to break out of phagocytic captivity into its target cell's cytoplasm. Besides mono-nuclear phagocytes, Listeria can infect liver and intestinal cells. Because it resides in the cells' interior, the bacterium seldom presents an antigen, so defies protection by the antibody arm of the body's immune system. That leaves T-cell response as the sole defence.

Cossart's team has cloned a fragment of the gene that codes for the LLO virulence factor in a shuttle vector, and tested it by transforming a non-pathogenic mutant strain of Listeria, making it virulent. By definition, LLO is specific to L. monocytogenes. (Extracted from Mc Graw Hill's Biotechnology Newswatch, 20 August 1990)

Industrial microbiology

Nitto biocatalyst for acrylamide

Continuing what it calls the first ever use of biocatalysts to make a commodity chemical, Nitto Chemical (Tokyo) will triple acrylamide capacity at Yokohama to 20,000 millions tons/year, using proprietary enzyme catalyst technology. Nitto's third-generation enzyme - Lodococus Lodoclose J1 - has higher tolerance of acrylamide than the second-generation enzyme it is now using, and can produce acrylamide directly, sidestepping the need for concentration. Solicited by US and European acrylamide producers, Nitto is studying whether to establish overseas production using the process, either alone or in joint venture, or to license it. (Source: Chemical Week, 27 June 1990)

Enzymes produced via genetic engineering

On the opening day of the Fifth European Congress on Biotechnology in Copenhagen, Steen Riisgaard, Head of Novo Nordisk's Bioindustrial Group, claimed that within a few years almost all industrial enzymes would be derived through genetic engineering.

Danish-based Novo Nordisk is the world's largest manufacturer of enzymes for industrial purposes, with a 50 per cent share of a world market estimated at $615 million in 1989. Riisgaard's claim follows the company's launch of the world's first detergent fat-splitting enzyme, Lipolase, which was produced using genetically engineered micro-organisms. Lipolase is now being manufactured on a commercial scale for the detergents industry.

Riisgaard also focused on the positive contribution that enzymes and biotechnology are making in the environmental field. Examples cited included an enzyme alternative to lime and sodium sulphide mixtures used as a hair remover in the leather industry, enzymes that can eliminate cyanide in industrial wastewaters; and, more recently, enzymes for the paper industry which will lead to substantial reductions in the use of chlorine bleach, another significant industrial pollutant. Details from: Novo Dordisk A/S, Novo Alle, 2880 Bagsvaerd, Denmark. (Source: Biotechnology Bulletin, Vol. 9, No. 6, July 1990)

AP expands biodegradable resins

Air Products and Chemicals has begun construction of a commercial-scale plant to produce its Vinex biodegradable thermoplastic polyvinyl alcohol resins. The plant, at Allentown, Pennsylvania, is due onstream by the end of this year.

The resins, claimed to degrade in the presence of moisture and the bacterium Pseudomonas boreopolis within six months, are targeted at applications such as agricultural films, blow-moulded containers for organic liquids and disposable personal-care products.

Air Products has been producing developmental quantities of Vinex resins since 1988.

The company produces polyvinyl alcohol at its plant at Calvert City, Kentucky. A second polyvinyl alcohol plant is due onstream at its Pasadena, Texas, complex in late 1991. (Source: European Chemical News, 1 October 1990)

Energy and environmental applications

Photobiotechnology offers fuel of the future

Biotechnology may have the solution to the world's energy crisis. "Harnessing solar energy will be the central feature of biotechnology in the future", according to John Pirt, emeritus professor of microbiology at King's College, London.

Although the main driving force of current biotechnology is exploiting genetic manipulation, particularly in pharmaceuticals, "the big future for biotechnology", Pirt believes, "must lie in the provision of renewable resources of energy, chemicals and novel foods".

Photobioreactors, which fix and transform one renewable carbon source, carbon dioxide, into reduced carbon chemicals using photosynthesis, are the key to this novel biotechnology. But, despite its great potential, photobioreactor technology has been "almost totally neglected", Pirt says.

"It is feasible to replace fossil fuel on a global scale by photobioreactor technology", Pirt asserts. The initial investment required to enter this field is "insignificant".

In a photobioreactor, micro-algae catalysts trap sunlight and use it to split water into oxygen and hydrogen. The hydrogen is then used to reduce carbon dioxide, obtained from the chemicals and brewing industries, cement manufacture or carbon fuel burning power stations, and synthesize biomass. The process is known as oxygenic photosynthesis. The resulting biomass can be converted into methane, ethane or other useful products, or it can be burned instead of fossil fuels.

Prototype reactors have been set up in Spain. They consist of arrays of transparent tubes of 1 cm diameter covering an area of 100 m^2. Originally they were based at Reading University where they produced 2 $g/m^2/h$ of biomass.

Photobioreactor technology offers many advantages over conventional agriculture. Arable land is not required, saline water can be used and there is no nutrient loss, for example. A photobioreactor can yield 50 t/ha/y of biomass compared with a crop yield of 10 t/ha/y for conventional agriculture. The area of photobioreactors required to produce 100 Mt coal equivalent is a square with sides of 182 km. Five of these areas would be needed to provide the total UK energy requirements.

The estimated cost of producing biomass in photobioreactors is around £800/t, with the major factor being the cost of the bioreactor itself. This compares favourably with the cost of producing food, but biomass is still too expensive for chemical feedstocks or energy production. However, costs could be decreased if the photobioreactor process is developed further. (Source: Chemistry and Industry, 17 September 1990)

Micro-organisms to degrade pollutants

A new biofilm has been developed as a reactor for the removal of volatile organic chemical pollutants from water, in research by Peter Wilderer at the Technical University of Hamburg-Harburg. Micro-organisms that can degrade the pollutants were obtained by seeding the reactor with sludge from a waste-water treatment plant. The reactor has a gas-permeable silicone rubber tubing that carries pure oxygen or carbon dioxide-free air. The test solution contained benzene, toluene and xylene (BTX) pollutants from the Georgswerder landfill in Hamburg. The BTX solution was held to a near-zero concentration of oxygen, so that the micro-organisms moved to the membrane tubing and formed a biofilm on the membrane. After four days, the micro-organisms were converting more than 90 per cent of the BTX pollutants in solution into carbon dioxide and biomass byproducts. With vigorous agitation in the reactor to knock off some of the biofilm thickness, the overall bioremediation rate was higher. The biofilm reactor eliminates the problem of escape of the volatile chemicals that arises when biodegrading micro-organisms are bubble-aerated to obtain oxygen. Future research will include developing a bioreactor to break down dibenzofurans using a Brevibacterium species. (Extracted from Bio/Technology, June 1990)

Bacteria-blend for complex waste

A newly-formed UK bioprocessing company is offering industries a novel, tailor-made mixture of bacteria to treat complex effluent streams.

Viridian Bioprocessing, based in Whitstable, Kent, offers the Microbial Custom Blend process, which uses a data base of several hundred different species of bacteria to find those that degrade each component of the mixture of effluents from a plant. The bacteria are then combined so that they work together to treat the complete effluent stream.

Some of the bacteria are taken from the plant site itself, where they have already been feeding and breeding on effluent and so come ready-designed. Many are also found at local sewage treatment works.

The company is now drawing up proposals for 20 companies in the UK, US, France and Germany and has had enquires from many more.

Viridian is now investigating bacterial blends that will degrade the most stubborn wastes - those that are highly chlorine-substituted. (Source: European Chemical News, 3 September 1990)

Rock crunching snails turn the desert green

Rock-eating snails may fertilize deserts with nitrogen, according to two researchers in the US. Clive Jones and Moshe Shachak have found that faeces from three species of tiny snails in Israel's semi-desert Negev region contribute a significant fraction of the soil's nitrogen.

The snails, of the species Euchondrus albulus, E. desertorum and E. ramonenis, are each less than a centimetre long. They feed at night on endolithic lichen - which lives inside the limestone rocks of the Negev. The snails use their abrasive tongues to get at the lichen, which can live up to seven millimetres inside the rocks. During the day, the snails rest under stones, and deposit their faeces.

Jones and Shachak calculate that each year the faeces contribute between 22 and 27 milligrams of nitrogen per square metre of soil, 11 per cent of the total input. A shortage of nitrogen can limit plant growth in deserts, so this provides an important boost to desert shrubs such as saltbush. Nitrogen inputs to the soil are often low, and small pools of nitrogen in the soil are easily drained by erosion and rain runoff.

The fertilising effect of the faeces may be magnified because of where the snails deposit them. Faeces left under a rock are also less likely to be washed away by rain.

The snail link between lichen and plants connects two distinct ecosystems. The lichen, catching airborne nutrients, stay in the rock while vegetation lives in the patches of soil which cover about 30 per cent of the Negev.

Without the snails, the nitrogen and other minerals absorbed by lichen would stay trapped in the rock, and not reach the plants. But at least 27 per cent of nitrogen trapped by lichen each year is passed on to the soil by the snails, according to Jones and Shachak.

Two alpine species that are closely related to the Negev snails may also be doing a similar sort of thing.

The snails also convert rock to soil at a rate of between 70 and 110 grams per square metre per year, which is equivalent to the eroding effect of windborne dust.

The harsh conditions of the Negev have made the snails-lichen ecosystem a simple one, suitable for studying in the laboratory.

The main source of water for both snails and lichen is dew rather than rain. The snails come out to feed in the desert only on nights when they sense a dewfall. (Source: New Scientist, 8 September 1990)

Carbon/membrane technology boosts biodegradability

A unique combination of activated carbon adsorption and membrane filtration is being used to step up the biodegradability of waste water leaving the Sandoz complex at Muttenz, Switzerland.

A new pre-treatment plant built by Uhde has come onstream to deal with the waste from the Muttenz complex, which produces chemicals for the paper and textile industries as well as agro-chemicals. The facility can deal with all the waste

from four large production units, corresponding to about 2,600 m^3/day on a five-day basis.

The plant uses an activated carbon adsorption process developed by Uhde in combination with Sandoz' own membrane filtration process. Smaller molecules are adsorbed by the activated carbon, while larger ones are separated by membrane filtration.

Dr. Pierre Meyer, assistant vice-president of dyestuff production at the Muttenz complex, said: "When the two processes work in parallel, they act synergistically to increase the biodegradability of waste water from about 65 per cent to the statutory value of 85 per cent. The technology is expensive however.

The activated carbon is regenerated in two steps. First, the carbon is washed to remove salt and dried on a double-bed oven at 600°C. Second, the carbon is roasted at around 850°C in a limited amount of air.

The plant designers also looked at the increase in biodegradability achieved using peroxide treatment with adsorption on activated carbon, but found that this was not as effective. (Source: European Chemical News, 10 September 1990)

Nitrogen emissions from biomass burning

Biomass burning has been shown to be important for atmospheric chemistry and for the biogeochemical nitrogen cycle of tropical savannahs and agricultural ecosystems. Using laboratory measurements of emissions from burning tropical vegetation, Jörgen M. Lobert and colleagues at the Max-Planck Institute for Chemistry in Mainz, Federal Republic of Germany, calculate that 12 million to 28 million metric tons of nitrogen are emitted each year from the burning of biomass. This is about 9 to 20 per cent of the nitrogen that is fixed by terrestrial vegetation each year. Lobert estimates that more than half of the biomass nitrogen is converted to molecular nitrogen. Their measurements indicate that about 13 per cent of the biomass nitrogen is converted to nitrogen oxides (NO and NO_2), about 4 per cent to ammonia, and about 3 per cent to hydrogen cyanide and acetonitrile. The remainder is emitted as other organic nitrogen compounds. The quantity of nitrogen oxides emitted is so great that it could account for about 20 per cent of the global budget, but very little nitrous oxide (N_2O) is produced. Because the amount of biomass burned each year is rising, it may be an increasingly important mechanism for returning biologically fixed nitrogen to the atmosphere. (Reprinted with permission from Chemical and Engineering News, 13 August 1990, p. 30. Copyright (1990) American Chemical Society)

Extraction industry applications

Bugs attack organic sulphur

Preliminary results have been announced in the US project to use bacteria to remove sulphur from fossil fuels before combustion.

Having already tested cultures successfully for the desulphurisation of coal, the microbes have now been shown capable of removing over 90 per cent of organically-bonded sulphur from various crude oils and distillates - and over 95 per cent in some cases.

The tests were performed by the Chicago-based Institute of Gas Technology which has licensed the technique to Environmental BioScience Corporation of Boston. IGT has developed a bacteria culture which specifically targets the C-S bond. Other methods are already available to remove free and inorganic sulphur, but organically-bonded sulphur has previously been impossible to remove prior to combustion.

All the work so far has been carried out on a laboratory scale. Pilot scale tests are loosely scheduled for 1992. Commercial availability should follow close on the heels of the pilot tests as this sort of microbial treatment is relatively simple to implement.

EBC microbiologist, Dr. Daniel Monticello, says: "The commercial application of this technology will represent one of the most significant impacts biotechnology can have on the environment in the 1990s". Successful application of this technology will release the vast world-wide high-sulphur crudes that currently remain in the ground because of environmental constraints. (Source: Process Engineering, September 1990)

Recovery of molybdenum, scandium by microbial action

Some yeasts and fungi out-perform ion-exchange resins in extracting scandium (Sc) from solution and efficiently adsorb molybdenum (Mo) as well, according to Gregory I. Karavaiko, Chief, Microbial Transformation of Minerals Laboratory of the Institute of Microbiology, Moscow, USSR. The metal-scavenging micro-organisms, he said, are about to be tried industrially, to recover these two valuable transition elements.

Sc occurs in association with aluminium ores. His bio-mass recovery process will be applied industrially at a bauxite plant in the Black Sea city of Nikolayev. Molybdenum extraction is to be tried at Balkashino, in Khazakstan, Central Asia.

In laboratory trials, he told a biohydrometallurgy symposium that "biomass of 20 strains of yeasts, bacteria and fungi sorbed Mo from solutions". In rapidity of adsorption, Rhizopus arrhizus (a common grain and fruit fungus) won, Candida scotti (fodder yeast) placed and Aspergillus niger showed. "R. arrhizus", Karavaiko stated, "combines a high sorption capacity with high affinity to molybdenum". It extracted approximately 170 mg of metal per gram of biomass.

He noted that "the mechanism of adsorption of Mo has not yet been elucidated. The cells of micro-organisms appear to adsorb mainly polymeric moieties of the metal". He also suggested that the bio-recovery process can be used industrially to separate Mo from tungsten.

As for scandium, Saccharomyces species was the star micro-organism, extracting 98.8 per cent of the rare-earth metal after four cycles of sorption. "The ability of micro-organisms to sorb rare earth metals", Karavaikov observed, "has so far remained virtually unstudied". In his experiments, "micro-organisms more actively sorp Sc ... and achieve a higher degree of extraction ... than ion exchange resins", the current industrial process. (Source: Mc Graw Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

E. PATENTS AND INTELLECTUAL PROPERTY ISSUES

Moore case judgement on removed body tissue

The decision of a California high court early in July that a patient has no rights to body tissue removed in an operation came as a great relief to the US medical community. The case had been brought six years ago by a patient, John Moore, who had a cancerous spleen removed in 1976, to treat hairy cell leukaemia.

Subsequently, he discovered that the doctor had discovered his cells were unique, had patented their genetic composition - and then sold the cell line for $3 million to a biotechnology company trying to develop cures for AIDS and cancer. But Mr. Moore won the right to sue the doctor for not telling him about his research. In future it may be that doctors will have to tell patients what they intend to do before operating, so the patient can negotiate his or her own fee with a drug company. (Source: Biotechnology Bulletin, Vol. 9, No. 6, July 1990)

Genetic fungicide

US-based company DNA Plant Technology has gained patent protection for transferring a gene for the expression of chitinase into crop plants. By breaking down chitin in fungal pathogens that attack fruits and vegetables, the company hopes the enzyme will prevent post-harvest rotting.

So far, scientists at DNAP have successfully introduced the gene and expressed chitinase in tomatoes, potatoes, lettuce and sugarbeet. Initial field testing of transformed plants is expected to be completed by early 1991, and DNAP expects products based on the technology to reach the market by the mid-1990s. (Source: European Chemical News, 23 July 1990)

Bionematicides patent

Mycogen Corporation has been granted a US patent for several strains of bionematicides developed to attack and kill animal and plant parasitic nematodes, but which are non-toxic to mammals, birds, fish and beneficial insects.

The bionematicides are based on the Bacillus thuringiensis bacterium, which produces proteins that the company claims are environmentally safe while at the same time extremely toxic to certain nematodes.

According to a company statement, the Mycogen products are preferable to traditional chemical nematicides, which may have adverse environmental effects, or to traditional plant breeding efforts to create nematode-resistant plants, which often require lengthy and complex development processes. (Source: Chemical Marketing Reporter, 10 September 1990)

New biodrug process patent

Celgene Corporation has been granted a patent for a method of producing pharmaceuticals and other biologically active compounds, which the company says is a major advance.

The system is effective on a broad class of amines, which includes cardiovascular, antibiotic and antidepression drugs, along with sweeteners and agricultural chemicals, the company says. It is estimated that 500 drug compounds in development are candidates for the Celgene technology.

Patent number 4,959,606 granted on 21 August 1990 by the US Patent and Trademark Office, is in the field of chirality. Many compounds come in two molecular forms which are mirror images of each other. Usually, one form delivers the desired characteristic, while the other form often causes undesired side-effects or has no effect. Conventional chemistry has had only limited success in making these isomers. Celgene's scientists say they have developed a biological process to make the preferred isomers with enzymes from naturally occurring bacteria.

The process offers a new, practical method of achieving high purity, which can translate into lower dose rates, reduced side-effects and increased efficacy, according to the company.

Celgene is working with a number of companies to demonstrate the applicability of its technology in medical care, and has developed a method to scale up its process to produce products in commercial quantity. (Source: Chemical Marketing Reporter, 27 August 1990)

New provisions regarding deposit of micro-organisms in China

The Chinese Patent Office has now clarified the provisions of Rule 25 of the Chinese Patent Regulations. Deposit of a micro-organism in a Chinese depositary is not required:

(a) Where the micro-organism is commercially available anywhere in the world before the priority date or before the actual filing date in China;

(b) Where samples of the micro-organisms were deposited in a depositary institution designated for the purpose of patent procedure (i.e. a Budapest Treaty deposit), and where the relevant micro-organisms were disclosed either in a granted patent or a pending application published before the priority date or the Chinese filing date.

A deposit is required where a micro-organism has not been deposited, or is deposited in an institution other than one designated for the purpose of patent procedure and not accessible to the public, or is not available to the public before the priority date or filing date. At the time of filing, it will be necessary to provide documentary evidence of the date of publication of a relevant application or of the date of grant in the form, for example of a copy of publication in a patent gazette, or documentary evidence of availability of the organism, for example listing in a commercial catalogue. Otherwise a sample of the micro-organism must be submitted for deposit at the time of filing. These provisions are effective from 6 June 1990. (Source: ABA Bulletin, Vol. 5, No. 4, August 1990)

New Zealand patent law under review

The New Zealand Ministry of Commerce, which administers patent legislation, is considering possible changes to the New Zealand Patents Act 1953, in order to provide harmonization at least with Australia. The area of medically-related inventions and biotechnology inventions will be particularly examined, and it is expected that the Government will be under pressure to increase the scope of protection available. There is also some suggestion that changes introduced will affect applications pending at that time. In particular, biotechnology practice is being reviewed in consultation with the New Zealand patent profession, and it is intended to produce a manual of practice

to be published as each part of the review is completed. It is expected that New Zealand will accede to the Budapest Treaty. A second area to be reviewed is the patentability of methods of medical treatment of humans, including cosmetic and diagnostic methods, which are presently not allowed. Second medical use claims are also presently not accepted, even when couched in the so-called "Swiss type form"; however, a number of hearings on this type of claim are pending.

The New Zealand Patent Office will now accept claims to fragments of naturally-occurring compounds, including peptides, although in some cases specific disclaimers, for example to peptides found in nature, may be required. The previous office practice regarded such fragments as being prohibited on the ground that they were found in nature. (Source: ABA Bulletin, Vol. 5, No. 5, August 1990)

US Biotechnology Patent Protection Bill

A Bill designed to stimulate development of biotechnology-derived drugs and to protect US biotechnology firms against foreign competition has been introduced into the US House of Representatives. The proposed legislation would give the International Trade Commission jurisdiction to exclude foreign products made by using components, such as recombinant host cells, which are the subject of US patents, and would also grant patent protection for a process that uses novel starting materials, even though the process steps themselves were not novel. This would put the US on the same footing as the European patent convention countries and Japan. (Source: ABA Bulletin, Vol. 5, No. 4, August 1990)

Sequence data in patent applications

The new rules for filing sequence data in the US Patent and Trademark Office will come into effect on 1 October 1990. Where a patent application discloses an unbranched sequence of either four or more amino acids or 10 or more nucleotides, it will be necessary, either in addition to or instead of the conventional disclosure of the sequence information in the specification, to present the sequence information in a specific form referred to as a sequence listing, using standard abbreviations and a standard format; in addition to a hard copy listing, each sequence listing must be presented in computer readable form, as a single file either on a diskette or a magnetic tape. The format is based on software adapted from GenBank's AuthorIn program, and is called PatentIn. The US Patent and Trademark Office is aiming at electronic filing of complete patent applications in the near future. (Source: ABA Bulletin, Vol. 5, No. 4, August 1990)

Australian law finds balance

The Australian federal Government has drawn the line at patenting human beings. But in a debate intended to amend patent law to accommodate innovations in biology, it agreed that some other forms of life should be subject to patent law.

The proposed amendment to the Patents Act of 1952 would have prohibited the patenting of living animals, plants and micro-organisms unless assessed by a committee consisting of geneticists, people specialized in ethics and representatives from consumer groups. This amendment was defeated largely on the grounds that it might prevent the patenting of genetically engineered vaccines.

Instead, a proposed alternative amendment that human beings and their biological processes for generation should not be patentable inventions was accepted.

Under this new bill, the patenting of living things will be solely at the discretion of the Patents Office staff without reference to parliament, bioethics committees, the public or the constitution.

But the Institute of Patent Attorneys of Australia, in a report for the House of Representatives Standing Committee on Industry, Science and Technology, takes an opposing stance and argues that "if the categories of patentable subject-matter are altered to exclude living organisms, industry and other research organizations in Australia engaged in legitimate and worthwhile research programmes would be seriously disadvantaged". (Source: Nature, Vol. 347, 27 September 1990)

HIV infection - haemophiliacs win right to sue

In what could prove to be a turning point in their long campaign for compensation, more than 900 haemophiliacs infected with the AIDS virus won a legal battle that will strengthen their argument in court against the UK Government over its role in importing contaminated blood products in the 1980s.

The haemophiliacs' victory gives them access to government records that could prove essential to their case. The records detail government policy on the procurement of blood products in the 1980s, and are likely to shed light on at least one of the chief allegations: that despite knowing about the risk of HIV contamination, the Government continued to import clotting factor from the United States rather than boosting domestic supplies.

The haemophiliacs also allege that the Government was slow to introduce heat treatment for domestically produced clotting factor and screen high-risk domestic donors after 1985. By then HIV had been established as the cause of AIDS and a blood test was available.

By 1985 about 1,200 haemophiliacs in the UK had become infected with HIV, largely from contaminated clotting factors imported from the United States. Of these, 962 are seeking compensation, 210 have developed AIDS and 140 have died. So far the UK Government has made ex gratia payments of £20,000.

Gaining access to records important to compensation claims has also proved difficult for haemophiliacs in the United States where the targets of legal action are pharmaceutical companies. Unlike government departments, the companies, which include Alpha Therapeutics, Highland Therapeutics and Armour Pharmaceuticals - all subsidiaries of international corporations - are not bound by the Freedom of Information Act.

So far, the handful of US haemophiliacs who have filed negligence suits against some of these companies have met with little success. In one notable case, a claimant in Georgia succeeded in convincing a jury, only for the jury's verdict to be reversed by a judge. A common stumbling block for US claimants has been proving that they can trace the source of their infection to any one manufacturer; every year a haemophiliac uses thousands of units of clotting factor, and these are unlikely to all come from a single company.

But a more fundamental challenge, and one facing haemophiliacs in the UK as well as the United States, is the difficulty of proving negligence when most infections occurred in the early 1980s before the cause of AIDS was known and a blood test was available.

Some haemophiliacs, and in particular those in Japan, argue that by 1983 - the year before HIV was established to be the cause of AIDS - there was already considerable evidence pointing towards non-heat-treated products as the source of infection. But Japanese health officials contend that when the haemophiliacs called for the introduction of heat treatment at that time the source of infection was far from clear. Last year about half a dozen of the 2,000 Japanese haemophiliacs thought to be infected with HIV filed negligence suits against the Government and pharmaceutical companies. (Source: Nature, Vol. 347, 27 September 1990)

F. BIO-INFORMATICS

Japan publishes booklet on advances in agricultural biotechnology

The Biotechnology Section of the Japanese Ministry of Agriculture, Forestry and Fisheries has completed an introductory booklet entitled "Advances of Agricultural Biotechnology". The publication summarizes recent progress and future trends of biotechnology in agriculture, forestry and fisheries in Japan. The booklet, with a number of photographs, presents recent results of research on plants, animals, and foods, including: orchid mass propagation, development of artificial vegetable seeds, and new varieties of Chinese cabbage; embryo transplantation in cattle, gene recombination in pigs, gynogenesis in flounder, size increase in triploid sweetfish, and multiple uses of the silkworm. Also covered are the development of cyclodextrin as a functional sugar, cell fusion technology of pomato (hybrid of potato and tomato), and brewing applications. (Source: Bio/Technology, Vol. 8, May 1990)

Genetic Engineering: A Perspective on Current Issues (DSIR Crop Research Report No. 137) by Darryl Macer

Genetic engineering is an exciting new science that promises dramatic answers to many of the world's environmental, agricultural, industrial and health problems.

However, because of the complexity of the new techniques, and their rapid development, information designed for the interested non-specialist has been scarce. This report brings together, in an easily read form, the latest information on the applications of genetic engineering, moral and ethical concerns, environmental safety issues and legal and regulatory progress.

Importantly, the publication offers a New Zealand view on the issues. Available from: Information Manager, DSIR Crop Research, Private Bag, Christchurch, New Zealand. Tel.: 64 3 252-511. Fax: 64 3 252-074. (Price: $US 18) (Source: DSIR Crop Research)

Public Attitudes to Genetic Engineering in New Zealand (DSIR Crop Research Report No. 138) by Paul K. Couchman and Ken Fink-Jensen

Genetic engineering is regarded as vital to the maintenance of New Zealand's comparative advantage in the production, processing and export of biological materials.

Not since the development of nuclear technology have the ethical and safety concerns about science been of such importance.

This report presents the results of a comprehensive survey of the public of New Zealand plus three specialist groups, to the issues involved in genetic engineering. The public, farmers, scientists and teachers were asked about their interest in science and technology, awareness and perceptions of developments in science generally and genetic engineering specifically, their attitudes to the use of genetically modified products and patenting of life forms.

The report is only the fifth such study completed and represents the most recent and comprehensive investigation of public attitudes to genetic engineering. Available from: Information Manager, DSIR Crop Research, Private Bag, Christchurch, New Zealand. Tel.: 64 3 252-511. Fax: 64 3 252-074. (Price: $US 18) Source: DSIR Crop Research)

Strong growth for safer, cheaper biopesticides

The use of living species to control agricultural pests looks set for strong growth as they are relatively cheap to develop and safe in use. They are, however, unlikely to challenge the market dominance of chemical pesticides during the coming decade.

In its latest technology impact report (Biopesticides - technology impact report T031, costing $US 1,750 from Frost & Sullivan), Frost & Sullivan forecasts that the most dramatic increases will take place in Bacillus thuringiensis (BT) toxin products, which are mostly inactivated spore preparations. BT toxins currently hold a large share of the biopesticides market in the US.

The report lists nine other microbial pesticides, but says that commercial introduction of other microbial agents for insect control are perhaps 10 to 20 years away, due to paucity of commercial R&D effort at present. Novel formulations of BT toxins are expected to expand their use.

Use of live insects to control pests will grow rapidly from its current tiny base. They will find a good outlet in agricultural greenhouses in the US, as they have already in Europe and Canada. Fungal and bacterial inoculants to promote plant growth and prevent pathogens should be commercialized over the next five to 10 years, as ways of applying them to seeds are developed. A similar schedule for market entry is expected for viral insecticide formulations.

Use of microbes as herbicides looks less promising. Recent developments of safer chemical herbicides, prospects for genetic engineering and concerns about microbe specificity should keep biological herbicide use at insignificant levels for the foreseeable future.

Despite their modest success to date biopesticides are highly appealing. Chemical products require $40 million to develop and five to seven years to register; biopesticides need only $2 million and one to two years for clearance. Safety of biopesticides is generally much easier to prove than safety of comparable chemical pesticides.

Other factors in their favour are that danger to farm workers is lessened and public concern over food residues reduced. Organic farming will provide a boost to biopesticide use.

The market for these products is currently dominated by Abbott Laboratories and Sandoz Crop Protection. Start-up biotechnology firms such as Ecogen and Mycogen have recently entered the market and are taking a growing share of the BT toxin market.

Biotechnology and materials abstracts up at CAS

Five-year data from Chemical Abstracts Service confirm what most already suspect: scientific activity in biotechnology and materials science has picked up dramatically in recent years. An analysis of the number of abstracts in certain subject sections of the CAS data base from 1984 to 1989 shows Section 3, Biochemical Genetics, has nearly doubled in total number of abstracts, a 92 per cent increase. The number of abstracts in Section 57, Ceramics, increased 70 per cent - 24 per cent from 1988 to 1989 alone. Other areas showing growth of more than 30 per cent during the period include immunochemistry (34 per cent); fermentation and bioindustrial chemistry (36 per cent); terpenes and terpenoids (36 per cent); unit operations and processes (48 per cent); essential oils and cosmetics (53 per cent); surface chemistry and colloids (34 per cent); radiation chemistry, photochemistry, and photographics and other reprographic processes (45 per cent); and electric phenomena (46 per cent). (Source: Chemical & Engineering News, 11 June 1990, p. 11)

Panos dossier on biotechnology and the third world

Biotechnology has the potential to alleviate the global food crisis and transform the face of medical science, concludes Miracle or Menace?: Biotechnology and the Third World, published last July by the Panos Institute.

The report foresees many benefits for the third world. Twenty-eight new vaccines could be developed over the next decade, for example. The world's forests could be expanded by cloning old, highly productive trees. The yield of cassava, the staple food crop in Africa, could be quadrupled. And rinderpest, which each year kills two million cattle in the third world, could be wiped out using a new vaccine created by an Ethiopian scientist.

Private monopoly of biotechnology through patent ownership threatens to widen the divide between North and South, concludes the report's author, Robert Walgate. The Chinese Government, for example, has granted exclusive rights to two US seed companies for a new hybrid rice variety capable of increasing rice production by up to 25 per cent. Despite its non-profitability in Northern markets, the technology is being withheld from developing countries. Details of the report, priced at £6.95, from: The Panos Institute, 9 White Lion Street, London N1 9PD. (Source: Biotechnology Bulletin, Vol. 9, No. 7, August 1990)

Report evaluates the safety of biotech-derived foods

The International Food Biotechnology Council (IFBC, Washington, DC) has issued science-based recommendations for determining the safety of biotechnologically derived foods.

The group, which consists of members of the International Life Sciences Institute and the Industrial Biotechnology Association, both of Washington, DC, sees no reason for instituting additional regulatory measures. Its report concludes that existing procedures can assure food safety, and that regulation of genetically modified food plants and micro-organisms can be patterned on existing laws and practices.

IFBC recommends acceptance if: nutrient levels do not differ significantly from traditional food; the natural toxicants normally present in small amounts are well within typical and acceptable ranges; and new constituents do not present an unacceptable risk at the levels of exposure anticipated. (Source: Bio/Technology, Vol. 8, August 1990)

Proceedings of the International Symposium on Molecular and Genetic Approaches to Plant Stress (New Delhi, February 1990)

The application of modern biotechnology to the solution of problems related to plant stress is of great interest to agriculturists in developing nations. Nowhere are the effects of plant stress, whether instigated by pests, diseases, or environmental extremes, so significant as in third world countries, for many of them are losing the battle to feed an ever-increasing population. The Government of India, via the creation of the International Centre for Genetic Engineering and Biotechnology (ICGEB) in association with the United Nations Industrial Development Organization, has taken the leadership in establishing programmes that will examine basic and applied aspects of the molecular biology of plant stress. The recent International Symposium on Molecular and Genetic Approaches to Plant Stress, held under the auspices of ICGEB at New Delhi, 14-17 February 1990, provided a strong signal of India's commitment to research in this area.

Meetings to discuss biotechnology and the third world tend to be extremely diffuse and to deal mostly with generalities. The ICGEB plant stress symposium at New Delhi provided a welcome change: research discussions were of the highest calibre and at the leading edge of the science. Perhaps the greatest benefit of this international symposium was the opportunity for Western scientists to interact with Indian molecular biologists, entomologists, plant pathologists, etc., whose work is not so well known in the West as it should be. Many of the contributions were impressive, particularly those by young scientists whose excellent training and dedication were very much in evidence.

The symposium followed fairly traditional lines: plant stress resulting from biotic agents (viral, fungal, and bacterial pathogens, and insect pests) and from abiotic agents (drought, salinity and heat stress). Interspersed, however, were sessions that dealt with the genetics of insect and pathogen resistance, methods for transformation and regeneration of rice and other tropical plants, and the application of RFLP analysis to tag the genes that are important in the plant's response to stress. In addition, discussions of traditional breeding techniques were well co-ordinated with those dealing with modern approaches to plant improvement, providing an excellent mixture of basic and applied research and there was substantial evidence that, for once, plant breeders and molecular biologists were communicating with each other.

The symposium dealt with many new and exciting developments in the molecular biology of plant stress and on related matters dealing with protoplast regeneration and transformation in rice, for example. Throughout the symposium there was an

atmosphere of optimism and enthusiasm, for there is plenty of evidence that progress in this area of research has been much more rapid than was foreseen even a few years ago. The first products of agricultural biotechnology, seeds of crop plants that carry resistance to broad-range herbicides, are likely to be available within the year. Equally promising are: (a) transgenic plants that express coat protein genes of plant viruses and are resistant to viral infection, and (b) transgenic plants that carry the gene coding for Bacillus thuringiensis (BT) toxin and that are resistant to a variety of lepidopteran pests. Also promising, but remaining at a greater distance, is the exploitation of induced resistance, i.e. the plant's response to biotic and abiotic agents. In particular, discussion of the signalling system that results in increased synthesis of aromatic compounds, lytic enzymes, and cell-wall strengthening proteins was particularly illuminating. Similarly, an up-to-date discussion of protease inhibitors as related to wound response and insect resistance indicated that possible applications to agriculture are not far behind.

That transformation of rice protoplasts with BT toxin is possible was discussed by Chinese scientists (Y. L. Fan and collaborators) and this is particularly exciting in view of recent success in the regeneration of entire plants from protoplasts of the rice cultivar IR54 by the group under Tom Hodges at Purdue University in the United States. Equally exciting is the recent progress in unraveling the nature of the tungro virus, the agent of one of the most damaging diseases of rice. This work was the subject of an elegant presentation by Roger Hull of the John Innes Institute in Norwich, England. The nature of this virus had remained unresolved for many years and how scientists tracked down the components responsible for the disease syndrome could be the subject of a good detective story. Two different particles, one isometric, the other bacilliform, are required for severe disease induction and leafhopper transmissibility.

The work at different laboratories in many parts of the world demonstrate the need for international collaboration for the solution of problems that primarily affect third world countries. The efforts of the Rockefeller Foundation to support work on rice protoplast regeneration, tungro virus, and bacterial blight of rice on an international scale is particularly noteworthy. The Delhi symposium made an overwhelming case for international collaboration in science.

The proceedings of the symposium are now available, free of charge and as long as stocks last, from the ICGEB, c/o UNIDO, P.O. Box 300, Vienna International Centre, Vienna, A-1400, Austria.

First world-wide guide to biotechnology marketing

The British Library has just published The Biotechnology Marketing Sourcebook, the first world-wide guide to biotechnology advertising and publicity information. This book provides detailed, up-to-date advertising information on over 250 publications concerned with life sciences, biotechnology, health care, biochemistry and laboratory supplies. Each entry gives contact names and addresses as well as information on circulation, subscription charges, format, contents, subject coverage and readership. The cost of the book is £30. Further information: Paul Wilson, The Science Reference and Information Service, British Library, 25 Southampton Buildings, London WC2A 1AW (Fax: 071-323-7930).

Plant biotechnology

The English-language literature providing information on Chinese biotechnology is extremely scant. Recently the Chinese Academy of Sciences has published an English-language proceedings* for the China-Japan Symposium on Plant Biotechnology. Sponsored by both the Chinese Academy of Sciences and Suntory Ltd., Japan, the symposium was held in Shanghai, 23-25 May 1989 and provided a good chance for both Chinese and Japanese scientists to discuss and exchange information on developments in plant biotechnology in both countries.

The proceedings comprised 28 papers and 23 abstracts presented at the symposium by distinguished scientists. The topics covered included plant genetic manipulation, protoplast culture and fusion, artificial seeds, production of secondary metabolites, regeneration and micropropagation.

Chinese Medical Journal. Journal of the Chinese Medical Association

The only Chinese national medical periodical in English, this journal introduces advances and research results in China's medical sciences and technology, serving as a tool for international academic exchange.

Distributed by Pergamon Press outside the People's Republic of China.

Biotechnology Guide Japan 1990-1991

Biotechnology Guide Japan is a unique refererce source which presents detailed information on more than 500 Japanese companies involved in the field of biotechnology including: contact names, organizations conducting research, description of co-operative ventures, annual revenue and profit, lists of recent patents, charts indicating partnerships and affiliations, current products and products under development, and much more. 1990, 591 pp., softcover, 0-935859-66-7, $25.

For more information, or to place your order, write to: Stockton Press, 15 East 26th Street, New York, NY 10010. Prepaid orders save shipping and handling.

Biotechnology in Japan Yearbook 1990/91: Markets and Research/Development

The Biotechnology in Japan Yearbook 1990/91: Markets and Research/Development, the first comprehensive data and analysis resource on biotechnology industry developments and market trends in Japan will be published early in November 1990. This 600-page report is based on data from Nikkei Biotechnology in Japan.

The Yearbook contains detailed data on market sizes and Japanese corporations' R&D activities, arranged by market segment. With well over 150 categories covered, from AIDS to flavourings to artificial seedlings, the Yearbook covers the fields of pharmaceuticals, diagnostics, chemicals, cosmetics, foods, agriculture, animal breeding,

* Proceedings of China-Japan Symposium on Plant Biotechnology. By the Chinese Academy of Sciences. Paperbound 7.2 by 10.2 inches. 123 pages. Hans Ys BioConsultants, The Chinese Academy of Sciences, P.O. Box 74006, Wuhan, Hubei 430074, People's Republic of China. $US 15.

marine biotechnology, biomass natural resources, bio-electronics, bio-engineering, and research equipment. It also contains special reports on Japanese government ministries' biotechnology facilities, industry guidelines, biotechnology patents, bio-finance and newly constructed research institutes. Price: $1,200. Further information may be had from Japan Pacific Associates, 467 Hamilton Avenue, Suite 2, Palo Alto, CA 94301, USA. Tel.: 415-322-8441 or Fax: 415-322-8454.

Bioreactor markets growing

A combination of events are driving the bioreactor business. This small but very important market-place in biotechnology reached estimated US sales of $76 million in 1989, having grown from some $70 million in 1988. More growth is expected in 1990, with sales reaching a combined total of $91 million.

A new report,* published by Business Communications Co., provides a detailed study of bioreactor technology and its more advanced developments. It covers the underlying technological issues, related work in materials research, the development of new products, changes in the market-place and the role of the suppliers. Updated market data on the product areas for which the future bioreactor products are expected are provided and assessments of the situations in Europe and Japan are given.

The market for conventional bioreactors in the US is projected to grow from $39 million in 1989 to $50 million in 1990, $67 million in 1992, $116 million in 1995, and $177 million in 2000. This represents a projected growth rate of 14.7 per cent per year.

Membrane bioreactors, by contrast, are a much smaller market that appears to be growing much faster. In fact, it is projected to be the fastest growing segment, at 25 per cent per year. Part of the reason is that the installed base is small, so adding to it can create the false impression that it is a hot market.

The speciality bioreactor segment was an estimated $6 million in 1989, projected to grow to $7 million by 1990, and $56 million by the year 2000. This growth is an average annual rate of 20 per cent per year.

The Third Epidemic: Repercussions of the Fear of AIDS by Panos Institute, published in association with the Norwegian Red Cross, 320 pp., £5.95, paperback

This most recent book from the Panos Institute is the first effort to synthesize experience of the "third epidemic" of reaction and response to HIV and AIDS.

In choosing a global perspective and in focusing on the social impact of HIV/AIDS, the Panos Institute has rendered a great service. It presents many examples of fear, prejudice and discrimination from different countries and varied socio-economic and political settings, with a mixture of technical accuracy and readability which characterizes the best of Panos' work. Yet the book manages to avoid oversimplification that would have compromised both its integrity and its value to the reader. For the villains - ignorance, fear and also prejudice - are not bound by any specific social, economic or political system.

The Third Epidemic has many additional merits. It underscores the importance of HIV/AIDS in Asia, where a major epidemic is emerging. This epidemic, presently centered in Thailand, Myanmar and India, has great importance for the future of Asia. In the chapters on Africa, South America, the Caribbean, Europe and Asia, The Third Epidemic allows local voices to speak.

Reference to various World Health Organization documents are informed and appropriate.

In presenting The Third Epidemic, Panos has once again rendered a service to the fight against AIDS. This new book is unique, and most helpful and stimulating to the general reader as well as to the "AIDS specialist".

During the past several years, the Panos Institute has made several important contributions to the global effort against AIDS. Its previous books, AIDS and the Third World and Blaming Others, its monthly publication and its series of seminars for both journalists and non-governmental organizations, have helped considerably to inform and mobilize awareness about the HIV/AIDS pandemic. (Source: New Scientist, 1 September 1990)

Proceedings of the European Workshop on Law and Genetic Engineering

The graphic symbol chosen for the European Workshop on Law and Genetic Engineering, which took place in Hamburg on 14 and 15 December 1989, a paragraph symbol in the form of a DNA sequence or, vice versa, a DNA sequence in the form of a paragraph symbol, reflects the two aspects the Workshop was focused on: genetic engineering on the one hand and its regulation by law on the other. The Workshop was co-organized by the Landesverband Bürgerinitiativen Umweltschutz of North-Rhine-Westphalia and the Heinrich-Böll-Stiftung.

During the last decade, genetic engineering has increasingly become a relevant topic not only for authorities but also for legislators at the European level as well as national level.

Whereas both parliaments and governments for a long time hesitated to regulate genetic engineering research and its application, the situation has undergone dramatic changes within a very short time.

The speed at which directives and regulations are being passed presently seems to conform itself to that of the developments in the genetic engineering research:

- At the European level directives concerning the contained use and the deliberate release of genetically engineered organisms have recently been passed.
- In the Netherlands similar regulations have been passed.
- In the Federal Republic of Germany the Government has prepared a Genetic Engineering Bill.
- In Belgium a rDNA Committee has been set up recently.

* The report "Changing Bioreactor Business" is distributed in Europe by RauCon GmbH, P.O. Box 1062, D-6912 Dielheim, Germany (Tel.: +49 (6222) 73562, Fax: +49 (6222) 74884 and costs $US 2,650 (plus $US 50 for postage and handling)).

These proceedings aim at providing the reader with a comprehensive view on the scientific background of genetic engineering and legislation within the EEC.

Further details available from Dan Leskien, Joachim Spangenberg, Landesverband Bürgerinitiativen Umweltschutz, NRW.

Computer access to ATCC biological Culture Collection Catalogues

The American Type Culture Collection (ATCC) now has the complete catalogues of bacteria and phages and algae and protozoa available through the ATCC ONLINE data base service. The ONLINE service is accessible through the CODATA Microbial Strain Data Network (MSDN).

Included in the catalogue information are strain descriptions, instructions for culture maintenance, media formulations, and special uses for specific strains. Data bases for the ATCC recombinant DNA materials collection and the ATCC/NIH repository of human and mouse DNA probes and libraries are also available through the ATCC ONLINE service. Other features of the service include: electronic mail, international microbial data bases, an international hybridoma data base, and electronic conferencing.

Information on subscribing to this service can be obtained by writing to ATCC/BIF, 12301 Parklawn Dr., Rockville MD 20852, USA. (Fax.: (301) 231-5826)

MSDN/DATASTAR link up

The Microbial Strain Data Network (MSDN) and DATASTAR have agreed to provide an electronic gate- way between the two services so that MSDN users have access to DATASTAR data bases directly from the MSDN menu, and at a discount. DATASTAR offers a wide range of data bases in the fields of biomedicine, biotechnology, chemistry and business. Users wishing to access the DATASTAR services must first register with DATASTAR to obtain an ID and password. All instructions for use and price list will be provided by DATASTAR on registration. DATASTAR are offering a special discount rate to MSDN users. We do not have a contact address or fax for DATASTAR, but MSDN can be contacted at the Institute of Biotechnology, 307 Huntingdon Road, Cambridge CB3 0JX, UK. (Fax: (0223) 277 605)

Molecular sequences in patents data base from Derwent

Known as GENESEQ, a new data bank available from Derwent is based on EMBL formats (for nucleic acids) and SWISS-PROT formats (for proteins). It includes all nucleotide sequences greater than nine bases and all peptides greater than three amino acid residues, and probes of any length. In addition there is bibliographic data, including names of inventors, titles and comments on the sequence.

The user may purchase the data bank and software for use on a VAX or SUN workstation, or alternatively subscribe to Intelligenetics - which will also undertake searches on a contractual basis.

Details from: Derwent Publications Ltd., Rochdale House, 128 Theobalds Road, London WC1X 8RP. Details about the equipment, software and time-sharing account from: Intelligenetics Inc., 700 East El Camino Real, Mountain View, CA 94040, USA. (Source: Biotechnology Bulletin, Vol. 9, No. 7, August 1990)

PROTEAN II PC package

Proteus Molecular Design Ltd. has announced that the PC package PROTEAN II is nearing the end of a successful beta test phase. PROTEAN II analyses, stores and manipulates protein sequence data and, in particular, predicts both secondary structure and the presence of epitopic sites (of major importance in combating viral infections). Results obtained by one test site are to be published shortly. The package will be sold world-wide through local agencies. (Source: Proteus News Release. September 1990)

"Artificial expert" trims permit preparation from months to minutes

A computer program, designed to slash field-release application-preparation time from half a year to less than an hour, will be available to researchers by autumn, according to the US Department of Agriculture's National Biological Impact Assessment Program. It is the first use of artificial intelligence in biotechnology regulation.

The program contains all federal forms and regulations, but does not cover state or regional laws. The European Commission, the Soviet Union and countries in South America have expressed interest in adapting this programme to their regulations.

Tentatively christened "Intelligent Form Generator", the program answers "well over 100 questions" by scientists who want to get a permit - including agencies to contact. Based on the scientist's answers, the computer will draft the application. The USDA is also creating instructional workshops. (Extracted from McGraw-Hill's Biotechnology Newswatch, Vol. 10, No. 15, 6 August 1990)

G. MEETINGS

January 1991

13-17 January Clearwater Beach, Florida, USA	IUB Conference on Nucleic Acid Therapeutics. Further information from Dr. Eric Wickstrom, Department of Chemistry, University of South Florida, Tampa, Florida 33620, USA

February 1991

20-22 February London, UK	First International Symposium on Immunotherapy of the Rheumatic Diseases. Further information from Professor G.S. Panayi, Rheumatology Unit, Division of Medicine, 4th Floor, Hunts House, UMDS, Guy's Hospital, St. Thomas Street, London SE1 9RT, UK

April 1991

8-11 April McCormick Place, Chicago, Illinois, USA	Environmental Technology Expo: The Complete Pollution Abatement and Control Exposition and Conference. Further information from Cahners Exposition Group, 1350 E. Touhy Avenue, P.O. Box 5060, Des Plaines, IL 60017-9990, USA

9–11 April Brussels, Belgium	Second EFFoST – European Conference on Food Science, Technology and Engineering Education. Further information from Prof. J. Lenges, CERIA, Avenue Emile Gryzon 1, B–1070 Brussels, Belgium
15–19 April Lisbon, Portugal	International Conference on Environmental Pollution. Further information from ICEP Conference Officer, ICTR Secretariat, 11–12 Pall Mall, London SW1Y 5LU, UK
18–20 April Singapore	FIA '91 – Food Ingredients Asia. International Conference and Exhibition. Further information from Expoconsult, P.O. Box 200, NL–3600 AE Maarassen, The Netherlands
22–25 April Ostend, Belgium	International Symposium on Environmental Biotechnology. Further information from The Secretariat, ISEB, c/o TI–K.VIV, Attention Ms. Rita Peys, Co–ordinator, Desguinlei 214, B–2018 Antwerp, Belgium
22–28 April Ghent, Belgium	FTI – International Exhibition on Micro–electronics, Biotechnology, New Materials and their Utilization. Further information from Stiching Flanders Technology International v.z.w., Josef II–straat 30, B–1040 Brussels, Belgium

May 1991

11–21 May Newcastle–upon–Tyne, UK	Course on biosensors and their application to biotechnology, medicine and the environment. Further information from The British Council, P.O. Box 88, Edgecliff NSW 2027, UK
13–14 May Milan, Italy	BIOTECH RIA '91. Meeting on the state–of–the–art and evolution of immunoassay: Laboratory automation and immunodiagnosis of allergy. Further information from the Organizing Secretariat, Clas International, Via Pace 8, 25122 Brescia, Italy
13–15 May Milan, Italy	BIOTECH RIA '91 – International symposium on biotechnology of growth factors: Vascular and nervous systems. Further information from the Organizing Secretariat, Clas International, Via Pace 8, 25122 Brescia, Italy
13–16 May Riva del Garda, Italy	13th International symposium on capillary chromatography. Further information from Professor P. Sandra, Laboratory for Organic Chemistry, University of Ghent, Krijgslaan 281, S4 B–9000 Ghent, Belgium
15–16 May London, UK	COSHH III – Control of substances hazardous to health environment safety – conference and exhibition. Further information from Labmate Ltd., Newgate, Sandpit Lane, St. Albans, Herts, AL4 0BS, UK
23–24 May Zurich, Switzerland	EURO FOOD TOX III – Effects of food on the immune and hormonal systems. Further information from Euro Food Tox III, MGB Zentrallabor, P.O. Box 265, CH–8031 Zurich, Switzerland

June 1991

3–7 June Basle, Switzerland	HPLC '91 – 15th International symposium on column liquid chromatography. Further information from Professor H. Engelhardt, Applied Chemistry, University Im Stadtwald, D–6600 Saarbruecken, Germany
5–7 June Orlando (Lake Buena Vista), Florida, USA	Biocatalysis for the 90s. Further information from Ms. Rita Kessel, Butterworth–Heinemann, 80 Montvale Avenue, Stoneham, MA 02180, USA
9–15 June Frankfurt–am–Main, Germany	ACHEMA '91. Further information from Dechema, P.O. Box 970146, D–6000 Frankfurt–am–Main 97, Germany
11–13 June Atlantic City, New Jersey, USA	Industrial and environmental laboratory conference and exhibition. Further information from Tower Conference Management Co., 800 Roosevelt Road, Building E408, Glen Ellyn, IL 60137–5835, USA
16–27 June Porto Hydra, Greece	Angiogenesis in health and disease (NATO Advanced Study Institute). Further information from Dr. Michael E. Maragoudakis, ASI Co–Director, Department of Pharmacology, University of Patras Medical School, Patras, Greece 26500

August 1991

4–9 August Jerusalem, Israel	15th International congress of biochemistry. Further information from The Secretariat, 15 IUB Congress, P.O. Box 50006, Tel Aviv 61500, Israel
20–26 August Moscow, USSR	AGROTECH – International exhibition of agricultural technologies and products. Further information from Glahe International KG, P.O. Box 800349, D–5000 Cologne 80, Germany

September 1991

16–27 September Kusadasi, Turkey	Recent advances in industrial applications of biotechnology. Further information from Dr. S. Suha Sukan, Director, Biotechnology Centre, Ege University, Gida Muhendisligi, 35100, Bornova, Izmir, Turkey

23-26 September University of Montreal, Montreal, Quebec, Canada	Biotechnologies and environment for a sustainable development. Further information from Ms. Diane Chalifour, Project Co-ordinator, University of Montreal, C.P. 6128, succursale A, Montreal, Quebec, Canada. H3C 3J7
24-27 September Leeds, UK	BIOTECH UK - First UK Biotechnology Conference of the British Co-ordinating Committee for Biotechnology. Further information from BIOTECH UK Information, c/o J. D. Bullock, University of Manchester, Manchester M13 9PL, UK

January 1992

19-24 January Miami, Florida, USA	The 1992 Miami Bio/Technology Winter Symposium: Advances in gene technology - feeding the world in the 21st century. Further information from The 1992 Miami Bio/Technology Winter Symposium, P.O. Box 016129, Miami, FL 33101-6129, USA
19-24 January Brasilia, Brazil	World Conference on the Environment and Development. Further information from Conference Services, United Nations, United Nations Plaza, New York, NY 10017, USA

H. SPECIAL ARTICLE

Vaccines for the third world*
by Barry R. Bloom

The third world is the place where three quarters of the people of this planet live, where 86 per cent of all births and 96 per cent of child and infant deaths occur. 1/, 2/ At both a national and a human level the diverse problems of the developing countries are of staggering proportions. Most have heavy foreign debt and, as a consequence, these countries now transfer to the developed countries more hard currency than they receive ($31.1 billion in 1987). 3/ Most are burdened by disease (see table 1); 4/ the reality is that millions of people are sick because they are poor, and poorer because they are sick. Some indicators of the quality of life of people of the 40 poorest countries are listed in table 2, and the trends have not been encouraging. Per capita income has declined over the past five years and the percentage of national budgets spent on health has been unchanged or has diminished for eight years.

TABLE 1 Diseases of the Third World that potentially could be prevented by vaccines*

Condition	Deaths per year (million)	Estimated episodes or incidence per year (million)
Respiratory disease	10	15
Diarrhoea	4.3	28
Tuberculosis	3†	10
Measles	2	67
Malaria	1.5	150
Hepatitis B	0.8	3.7
Meningitis	0.35	1
Schistosomiasis	0.33	10
AIDS	0.1‡	0.75
Worm infections	<0.06	4,900

* Modified from J.A. Walsh (ref. 4)
† Data from WHO Tuberculosis Unit
‡ Data from WHO, unpublished estimates

TABLE 2 Indicators of the quality of life in the world's 40 poorest countries, 1988

Average annual gross national product per capita	$270
Literacy	
Males	42%
Females	21%
Population with access to water	
Urban	61%
Rural	21%
Government expenditure	
Health	5.5%
Education	14%
Defence	13.8%
Population per physician	6,050
with access to health services	40%
Life expectancy at birth	48 yr
Infant mortality (<1 yr per 1,000 births)	130
Mortality rate of children aged under 5 (per 1,000 births)	211
Population annual growth rate (1973-84)	2.6%
Children suffering malnutrition	31%
Children immunized with DPT, polio, measles and BCG	61%

Yet one aspect of life there has improved profoundly. The number of children receiving immunizations has risen from 5 per cent in 1974 to over 60 per cent this year. The World Health Organization Expanded Programme for Immunization (EPI) prevented the deaths of 2.2 million children last year. Through the efforts of 25,000 professional national and international staff and hundreds of thousands of field workers, 60 million children are now vaccinated annually against diphtheria, pertussis, tetanus (DPT), polio, measles and tuberculosis. The number of cases of paralytic poliomyelitis has declined in the Americas from 4,500 ten years ago to fewer than 200 this year, and WHO has just made the eradication of polio one of its goals. 5/ Immunization is the most

* This article, which is reprinted with the permission of the author and publisher, first appeared in Nature, Vol. 342, 9 November 1989.

cost-effective weapon for disease prevention in developing countries, and new molecular and genetic technologies are making new types of vaccines feasible. The eradication of smallpox demonstrated that they can be effective everywhere. What is lacking is the will to make the advances of modern biomedical science available to the poorest people of the world.

Vaccine problems and constraints

As impressive as the results of global childhood immunization have been, there are many diseases for which vaccines are not available, as well as inherent limitations in each of the currently used vaccines. Only two EPI vaccines - BCG, used to immunize against tuberculosis, and oral polio vaccine - can be given at birth or any time thereafter, and BCG and measles are the only vaccines that require a single immunizing dose. DPT and polio must be given several times to achieve protective levels of antibodies. Because maternal antibodies circulating in the infant inactivate the vaccines, DPT can be given only at six weeks of age, and then at monthly intervals up to 14 weeks. The dropout rate is high - about 20 per cent fail to return for each required booster shot. In the case of the vaccine against measles, which is responsible for the death of 2 million children annually in the developing world, the presence of maternal antibodies that neutralize the vaccine in young children represents a "wall" that scientists have not yet been able to hurdle. If measles vaccine were given to children in developing countries at 12-15 months, the time recommended in the developed world, up to 30-50 per cent would have contracted the disease before receiving the vaccine. Measles vaccine is given at 9-12 months and a new strain may even be effective at six months. In all, a total of five contacts are required between the child and the health services, which represents a considerable logistical problem.

Any new or improved vaccine to be considered for inclusion in the EPI should, ideally, have the following attributes: (1) it must be inexpensive; (2) it must be safe; (3) it must be extremely effective, inducing protection in 90-100 per cent of recipients; (4) it should engender lifetime immunity; (5) it should be heat-stable and not need to be kept cold at all stages (a cold chain), which is both expensive and subject to catastrophe; (6) it should require only one shot or be compatible with the schedule for other vaccines; (7) it should be simple to give, and because of the problems of reuse of needles and of HIV infection it should be given by a non-invasive route; and (8) it should be capable of being given as close to birth as possible. Dr. W. Foege, Chairman of the Task Force on Child Survival, has described the ideal vaccine as one in which "single administration at birth will provide protection from multiple diseases".

New vaccines from old

There are basically four kinds of vaccine, each of which has strengths and limitations for use in the third world:

Killed vaccines are among the simplest and least expensive to prepare. They contain many antigens and assure some responsiveness in virtually all individuals. But even under defined production conditions, some vary in reproducibility, and all require careful monitoring to assure that no viable organisms are present. As in the case of pertussis, killed vaccines often have a higher level of reactigenicity or toxicity than is desirable.

Subunit vaccines prepared from individual components of a pathogen, by chemical synthesis or recombinant DNA technology (for example tetanus and diphtheria toxoids) have several advantages. They are chemically defined, reproducibly prepared and assayed, and are usually inexpensive to manufacture. Carbohydrate antigens are important for protection against many pathogens. Because complex carbohydrates cannot be produced by recombinant DNA technology, subunit vaccines, particularly carbohydrate-carrier-protein conjugate vaccines, are the only feasible strategy at present for producing protective immunity to pathogens such as Haemophilus influenza b and Streptococcus pneumoniae, which cause meningitis, mental retardation and pneumonia.

A general drawback of both killed and subunit vaccines is the need for several immunizations and boosters - immunological memory benefits from repeated stimulation by antigens. Some chemically defined subunit or peptide vaccines contain a desired antigenic determinant (epitope) that elicits B-cell-produced neutralizing antibodies, but they may not engender immunological memory to the infectious agent which requires expansion of specific T-helper cells as well; thus reinfection will not result in a boosting of the immune response. The possible use of small synthetic epitopes in subunit vaccines raises the concern that polyclonal antibodies developed against a single epitope of a pathogen may function very much like monoclonal antibodies and select for escape mutants - that is, antigenic mutants with altered epitopes on neutralizing antigens that are not neutralized by existing antibodies in the population. Although this would not be catastrophic for a single epitope in the first instance, new antigenic mutants could be created that would, in time, accumulate and possibly become resistant to normal acquired immune defences.

Live attenuated vaccines have largely been derived by empirical methods, and those in current use have the advantages of generally inexpensive production, persisting immunity and a good safety record. Nevertheless, there are considerable problems with existing live vaccines in terms of reversion to virulence (polio, Sabin type 3), reactigenicity (BCG), need for a cold chain, and possible induction of disease in immunodeficient and some normal individuals (for example polio).

Anti-idiotype vaccines are a novel concept that has yet to be realized. They are based on the principle that because antibody active sites (the idiotype) are complementary to the specific antigenic determinant to which they bind, some antibodies raised against a particular antibody active site may indeed mimic antigen. Although immunization has been achieved in some experimental systems with anti-idiotype or anti-antibody vaccines, so far there has been difficulty in achieving high levels of immunization and immunological memory because T cells are not developed against antigens of the pathogen. It remains to be seen in what circumstances anti-idiotype vaccines will prove to be useful.

In considering the various criteria for new vaccines for the third world, genetically engineering live attenuated vaccines to become multivaccine vectors that can immunize simultaneously against multiple antigens is particularly appealing. Two basic strategies are being developed, one using viral vaccines, the other using bacterial vaccines as recombinant multivaccine vehicles. Viral vaccines in general have certain

advantages: they express antigens in eukaryotic cells with correct folding, proteolytic processing, glycosylation, secretion and subunit assembly; and they can stimulate the production of cytotoxic T-lymphocytes as well as antibodies. Bacterial vaccines have a special ability to immunize for long periods and engender local immunity, for example in the gut, and cell-mediated immunity.

The technology: the power of molecular biology and genetics

The ability to change living organisms by genetic engineering has given rise to the possibility of devising vaccine vehicles that can immunize simultaneously against antigens of different pathogens. Such multivaccine vehicles can be developed from either viral or bacterial vaccines, and each approach has its particular advantages.

Live attenuated viral vaccine vehicles. Smallpox was eradicated by the use of live attenuated strains of vaccinia virus, originally derived by Jenner from a cowpox virus. Although vaccinia may be of somewhat dubious provenance, it has been astoundingly effective. The virus is a large double-stranded DNA virus of about 185,000 base pairs that contains all the information for its own transcription and replication in the cytoplasm of a wide range of host cells. Large amounts of DNA, approximately 25,000 base pairs, are expendable and not required for vaccinia replication. The molecular strategy to develop vaccinia into a multivaccine vehicle is based on the ability to replace non-essential viral DNA with foreign genes, and have them expressed under the control of the vaccinia-virus promoters and transcription system. Plasmids have been constructed containing one or more promoters, cloning sites for introducing DNA for foreign antigen genes, and a selectable marker, all flanked by DNA sequences that complement segments of non-essential DNA of the virus. When cells containing the plasmid are infected with vaccinia virus, a low frequency of double recombinations can occur between the flanking DNA sequences surrounding the foreign DNA in the plasmid and the non-essential DNA of the virus, resulting in the precise replacement of the viral DNA sequences by the desired promoter and foreign antigen gene. A wide range of viral, bacterial and protozoal antigens has been expressed in vaccinia, as many as four antigens being expressed simultaneously in a single recombinant virus. 6/

The principal disadvantage of vaccinia is the frequency of complications, which approaches the limits of current acceptability. Although only one in 300,000 recipients of the least troublesome vaccinia strain developed severe neurological effects, the incidence of serious complications, including disseminated vaccinia and vaccinia gangrenosum, was of the order of one in 1,000 in New York State, and is certainly higher in some developing countries. 7/ (It is regrettable that data on complications in developing countries are not available from the global eradication campaign.) By genetic engineering it should be possible to define viral genes involved in neurovirulence and reactigenicity and to create new, less reactigenic, vaccine strains. It has also been possible to incorporate genes for lymphokines such as interleukin-2 to enhance immune responses to foreign antigens and the virus itself.

Because recipients produce neutralizing antibodies to the virus, a second limitation of vaccinia is that it is not feasible, at least in the short term, to give booster shots, because reintroduced recombinant vaccinia will simply be neutralized. Practically speaking, vaccinia is a one-shot vaccine by necessity, and that shot must be highly effective to make it useful. Recombinant vaccinia expressing the glycoprotein antigen of rabies virus is extraordinarily immunogenic and, despite some uncertainty about vaccinia's acceptability in human beings, recombinant vaccinia has recently been introduced in Belgium, and a region of France, in bait to protect foxes against rabies. More importantly, perhaps, there is an epidemic in West Africa of rinderpest, a cattle disease caused by a measles-like morbilla-virus, and the International Commission of Epizootics plans to immunize hundreds of millions of cattle with recombinant vaccinia expressing rinderpest antigens. In an experiment of that scale, with perhaps 100,000 vaccinators, there is bound to be adventitious infection of human beings with the recombinant vaccine. It will be essential that they are pre-immunized against vaccinia virus.

Adenovirus vaccines have been used in tens of millions of US military recruits to protect against respiratory disease, essentially without serious complications. Curiously, the vaccine is not an attenuated strain, but rather consists of two virulent strains, types 4 and 7, that are given by an unnatural route, orally, that immunizes without producing disease. The use of enteric-coated lyophilized vaccine tablets represents a distinct advantage for delivery. The virus has a rather small DNA genome, but a limited number of foreign genes have been introduced and expressed in adenoviruses by a similar, precise gene-replacement strategy, including the hepatitis-B surface antigen that is not well-expressed in vaccinia. 8/ Concerns include the role of the E1A gene, which functions like certain proto-oncogenes in transformation of cells in culture, and the fact that the safety of existing adenovirus vaccines in children is unknown. Herpes viruses are large DNA viruses that, like vaccinia, have large amounts of expendable DNA; they could be developed into recombinant multivaccine vehicles, provided that the genes for neurovirulence can be identified and deleted.

Two RNA viruses offer promise as recombinant vaccine vectors. The live attenuated polio vaccine is one of the most effective vaccines in the developed world, but it has been disappointingly ineffective in many developing countries with up to 30 per cent of recipients failing to produce acceptable neutralizing titres, generally to type 3. There has been a return to multiple injections of killed polio vaccine in some places. Polio vaccines are not without problems, even in the developed countries, because essentially all cases of poliomyelitis there result from reversion of vaccine strains to virulence. Because Sabin type 3 vaccine differs from virulent poliovirus type 3 by only two mutations, reversion to virulence is an intrinsic problem.

The key to the genetic manipulation of polio was the discovery that artifically produced complementary DNA to the viral RNA is infectious and, on transfection of cells, produces infectious poliovirus. Of the several neutralizing epitopes on polioviruses, all but one are conformational and composed of discontinuous sequences of amino acids, the exception being a single loop of linear sequence. 9/ Several laboratories have been able to recombine cDNA sequences of the loop that generate the main neutralizing epitopes, for

example, from type 3 into the very stable polio type 1 vaccine, to create hybrid vaccines that generate neutralizing antibodies to both types 1 and 3 poliovirus. 10/, 11/, 12/ The cDNA sequences encoding the loop can be modified to express sequences for small epitopes of foreign antigens, including HIV. At present, however, there is no evidence that these recombinant vaccines can immunize by oral administration.

One of the safest and most effective live attenuated viral vaccines known is the 17D vaccine against yellow fever. A single immunization results in sustained neutralizing titres for periods of over 40 years, and complications are very rare. It has recently been possible to produce infectious cDNA from the 17D vaccine 13/ and one hopes that the possibility of developing 17D as a recombinant vaccine vector will be rapidly explored.

Live attenuated bacterial vaccine vehicles. The first modern approach to a rationally designed live attenuated vaccine was the production, by chemical mutagenesis, of an auxotrophic mutant of the virulent bacterium Salmonella typhi. A mutant in galactose epimerase (GalE) was selected that could not convert UDP-galactose to UDP-glucose. The strain, Ty21a, retained the ability to infect after oral administration and to colonize the gut, essential for producing secretory immunity. The mutation was designed to be a time-bomb; Ty21a can grow for several days, and then, when it accumulates more UDP-galactose than it can tolerate, it dies, liberating antigens. This strain has proved to be remarkably stable and safe, and has been tested in field trials in Egypt and Chile against typhoid fever.

Ty21a suffers from two disadvantages. One never knows precisely what gene(s) is affected by chemical mutagenesis. Indeed, when the GalE gene from S. typhi was deleted by another group and the deleted strain was tested in volunteers, two out of the four recipients developed typhoid fever, revealing that the key attenuating mutation in Ty21a must have been in a gene other than GalE. Equally distressing, three oral immunizations of Ty21a were required to achieve 67 per cent protection, an efficiency insufficient for a useful anti-typhoid vaccine or multivaccine vehicle.

To avoid the vagaries of chemical mutagenesis, the genetic strategy for salmonella vectors is to delete genes required for pathogenesis or survival, then to insert foreign genes into the chromosome by gene replacement, or express them on plasmids in the cytoplasm. A promising set of targets for gene deletion has been genes (aroA, C and D) of the aromatic-amino-acid pathway required for synthesis of folate and enterochelins (iron-binding proteins) as well as proteins. 14/ These are auxotrophic mutants that cannot grow without added aromatic amino acids, and thus they too function as time-bombs. Vast amounts of foreign genetic material can be introduced and expressed in bacteria, and these salmonella auxotrophic mutants have immunized animals against introduced recombinant antigens.

A second genetic strategy used to produce recombinant Salmonella typhimurium vectors has been deletion of the genes for adenylyl cyclase (cya) and the cAMP-binding protein (crp - catabolite repressor protein) which, together with removal of a plasmid containing virulence genes, has eliminated pathogenicity. Genes for foreign antigens expressed on plasmids introduced into these strains have persisted and immunized mice. 15/ The great advantage of salmonella recombinant vectors is that they can be given orally and, in experimental animals, they immunize both locally and, at the T-cell level, systemically.

Unfortunately, the few trials in human volunteers with recombinant salmonella vectors have indicated that the strains tested have been overattenuated and do not produce adequate secretory IgA antibodies. Patient efforts are required to construct salmonella strains that can achieve the delicate balance between appropriate attenuation and effective colonization leading to immunization. They must immunize not only against typhoid but also against introduced recombinant antigens; of particular interest are those for cholera, shigella and enterotoxigenic E. coli.

The oldest and most widely used live attenuated vaccine in the world is, perhaps surprisingly, BCG - Bacille Calmette-Guerin - an attenuated bovine tubercle bacillus used to immunize against tuberculosis. This mycobacterial vaccine has been given to over 1.5 billion people with a low frequency of serious complications, although it has the highest rate of minor reactions of any of the EPI vaccines. Its effectiveness in protecting against pulmonary tuberculosis has varied greatly in different parts of the world, but BCG requires only a single shot to engender cell-mediated immunity for periods of 5-50 years. It is known to be an effective adjuvant, enhancing immune responses to many different antigens. BCG can be administered at birth or at any time thereafter, and can be given repeatedly. Like most bacterial vaccines, it is very inexpensive ($0.055 per dose).

In contrast to E. coli, BCG grows very slowly and requires about 24 days to produce a colony (E. coli takes eight hours). Because it seemed unlikely that direct genetic manipulation of mycobacteria would prove efficient, a "shuttle" strategy has been devised to introduce and express foreign genes in BCG. Basically, phages or plasmids were constructed that can replicate both in E. coli and in mycobacteria. 16/ Foreign genes and selectable markers can be introduced by standard molecular genetic techniques in E. coli, and the recombinant DNA is then "shuttled" into BCG. Foreign genes can then be expressed either by replacing non-essential BCG chromosomal genes or from multicopy plasmids in the cytoplasm. It remains to be seen, however, how effective antigens known to be immunogenic when mixed with mycobacterial adjuvants are when expressed by the organism itself.

The spectre of AIDS

There is a serious risk with any live vaccine, namely dissemination and serious complications in immunodeficient individuals. The current HIV epidemic in many countries heightens concern about side-effects from childhood vaccination. For example, vaccinia has been administered to US military personnel, 28 of whom were later found to be HIV seropositive. One died of generalized vaccinia. (One cannot help but wonder, as by convention, study or use of smallpox (variola) virus has been discontinued and all strains presumably locked away in three safe repositories, why anyone in the world is being vaccinated against smallpox.) Several major cohort studies are under way in Africa to evaluate the consequences of standard childhood vaccination in HIV-seropositive compared to HIV-seronegative children. It is encouraging that

so far no discernible differences in untoward complications of any of the vaccines have been observed. It may be that there is a window in time in congenitally infected children during which they can be immunized and before which any significant immunodepression occurs. Because children who will be immunocompromised are even more susceptible to the principal childhood diseases, particularly measles, it is strongly recommended by WHO that these children receive all the childhood vaccines, except for BCG in children with symptomatic immunodeficiency.

The basic premise of existing vaccines has long been the utilitarian principle of providing the greatest good for the greatest number of people; it has long been recognized that small numbers of individuals will suffer severe complications. At the very least, antidotes to restrict disseminated infection of recombinant live vaccines should be available (for example, vaccinia immune globulin, isoniazid for BCG, antibiotics for salmonella). Because of the high cost of drugs potentially useful against AIDS, it is sadly likely that most will not be available to patients in developing countries in the foreseeable future; consideration of cost effectiveness alone should spur efforts to develop effective vaccines against AIDS.

Single-dose, controlled-release vaccines

The requirement for several injections is the main limitation to subunit vaccines. Almost 800,000 neonates per year still die of tetanus, which can be prevented simply by immunizing women of childbearing age. Controlled-release systems are already being used in man (and cattle) to deliver an array of drugs and hormones. Some of these systems have been used by the WHO Special Programme on Human Reproduction (HRP), which has supported research on anti-fertility vaccines. Taking advantage of HRP expertise in the area, development of single-dose vaccines has become a goal of the WHO Special Programme for Vaccine Development with neonatal tetanus as its first target.

Basically, polymers of lactic and glycolic acids (PLA/PGA) approved for human use are being incorporated into controlled-release microspheres or microcapsules which can provide either continuous release of tetanus toxoid over a period of several months, or a pulsed release similar to booster shots, that would occur, for example, 4 and 8 weeks after a single immunization. Should this prove effective, DPT, and a number of other subunit antigens could be incorporated into this type of one-shot vaccine - not the least important of which will be epitopes from human chorionic gonadotrophin or sperm to control fertility. 17/

Immunological problems

At a scientific level, the constraints on development of new vaccines are not likely to come from molecular biology and genetics; rather they will be biological and immunological. Although antigens have been cloned from virtually every known pathogen, and many T and B epitopes have been mapped on various antigens, there are very few pathogens for which the mechanism of protection is understood. Nor is it clear which antigens are required to engender those protective responses.

A multitude of unanswered questions remain. Must a protective vaccine against malaria sporozoites generate antibodies, or T cells, or both? Can production of specific immunoglobulin isotypes be targeted (for example IgA for intestinal pathogens, IgE for killing schistosomes, IgG for carbohydrate antigens)? Can the type of T-cells (for example helper T-cells, cytotoxic T-cells, gamma-delta T-cells, and lymphokines) produced be controlled (for example, by targeting the vaccine to the cytoplasmic compartment or endosomal compartments of antigen-presenting cells)? Can T-cell responses against important protective antigens be generated in individuals of all histocompatibility types? Can non-varying epitopes be found on highly polymorphic protective antigens that can be effective against genetic variants of the pathogens (for example malaria, HIV, African trypanosomes)?

At the same time, immunology offers novel solutions for basic vaccine problems. Measles vaccine cannot be used in children under the age of 6-9 months because it is inactivated by maternal antibodies, but it is conceivable that appropriate measles T-cell epitopes could be given at birth in one of the recombinant vaccine vectors, such that T-cell memory is generated to measles and a protective response would follow natural infection. Clearly, the easy vaccines have already been made; new vaccines pose greater challenges for research.

"It is only a matter of implementation"

There are few more portentous words than these to be found in any health document. For most scientists engaged in the development of new health interventions, the fulfilment of their research is a product that goes through clinical testing and is eventually licensed for use. In international health, the historical record belies such optimism. For example, all of the vaccines in the EPI programme were available before 1974, but only 5 per cent of the world's children received them - it was clearly only a matter of implementation.

Certainly, the main limiting factor is cost, but it is not the only one. The scientific infrastructure for evaluating new drugs and vaccines relevant to third world diseases is also limiting. It is difficult and expensive to carry out clinical trials in industrialized countries in which the diseases do not occur. From the beginning of the WHO-Tropical Disease Research (TDR) programme a component was mandated in addition to the scientific research programmes for "institutional research strengthening" in the countries worst afflicted. This support, representing 25 per cent of the budget, has been used to set up and modernize laboratories, and to train bright students abroad (and, occasionally, even to make it sufficiently attractive for them to return home). As a consequence, in many developing countries there are laboratories that are able to tackle tropical diseases.

What has been largely overlooked, however, is the role of field and epidemiological research in developing countries. Almost all incentives - financial, working conditions and personal recognition - motivate people to go into medical practice, laboratory-based research or, most commonly, administration. There are few rewards for health workers in the field. Yet the field worker is the mainstay for acquiring information about local health problems, for evaluating new interventions and for integrating, maintaining and monitoring them in control programmes. Recognition of their importance through the development of appropriate career structures and educational and material incentives is a major challenge to the developing countries.

At the same time, the development of new drugs and vaccines (38 new products that have resulted from the WHO-TDR programme are currently in field trials) provides a unique opportunity for building scientific and field infrastructures in the third world. Field research and control infrastructures need no longer be tied directly to one drug or vaccine, but can be continuing mechanisms to assess different health strategies and disease-control activities. Much of the same technology - enzyme-linked immunosorbent assays (ELISA) or polymerase chain reactions (PCR), for example - can be used in the same place to assess the distribution of parasites in mosquitos and the incidence of multiple infections by multiple pathogens in the population. The epidemiological tools for testing different drugs against one disease are often applicable to trials against another and can be adapted to evaluating vector control and vaccines as well. The result of this research is not papers; it is the control of disease.

A vital aspect of implementation has almost invariably been ignored. Fifteen years ago it became clear that although oral polio vaccine is superbly ineffective in developed countries, it was proving disappointing in developing countries. Studies show that only 40-80 per cent of children receiving oral polio vaccine produce antibodies sufficient to ensure protection. To this day, it is unclear why oral polio vaccine is so ineffective in developing countries. Is it that children are already colonized with enteric viruses that have a competitive advantage over the vaccine, or that maternal antibodies neutralize the vaccine? Or is it simply that the vaccine sold in the United States contains six times more viral particles?

Until two years ago, EPI's mandate was "implementation" only, and it lacked a research component. The tragedy, measured in lost lives, has been misapprehension that there can be implementation without research. In this context, I mean not only laboratory-based research, but social and economic research to provide information on costs and benefits, and to provide the means to understand how best to design and implement control programmes and identify and overcome obstacles to their effectiveness.

The main trend in development since the Second World War, as the Nobel prize-winning economist Theodore Schultz pointed out, 18/ has been investment in physical capital - dams, factories and so on - with little recognition of the potential for investment in "human capital". The role of health, education and quality of life in development has generally been underestimated, because it is much easier to measure direct industrial and agricultural productivity than the indirect political and economic effects of health and knowledge on the economies or political stability of developing countries.

Vaccines are clearly not the key to development. Yet they can and do serve as an entry point both to strengthening science and primary health-care infrastructures. The infrastructure for vaccines can be extended to other low-cost/ high-impact medical technologies on a mass scale, including oral rehydration, and maternal and child care and education, especially breastfeeding, food supplementation and family planning. In 1984, for example, Colombia vaccinated three quarters of its children under five years of age through three national immunizations days, and has subsequently mobilized complementary efforts for primary health care and education.

Much ink has been spilled on "appropriate technology" for the third world, but there has been almost no consideration of "appropriate science". Research on vaccines involves a knowledge of molecular biology, genetics, immunology and epidemiology, and could serve as a stimulus to the educational and scientific advancement important to development. Even the poorest countries have need for expertise and access to biomedical science; they cannot afford to squander their resources on iron lungs.

The bottom line

It is ultimately, one supposes, a matter of priorities. The annual budget for the entire World Health Organization is $327 million. The TDR Programme for six tropical diseases has a budget of $30 million, and the budget for the Special Programme for Vaccine Development has a budget of $2.8 million. The annual cost of childhood immunization is some $500 million, averaging approximately $10 per immunized child. The vaccines themselves represent less than 10 per cent of the cost of immunization; of the remaining costs for personnel, transport and logistics, 70 per cent is borne by the countries themselves. The major advance in vaccine coverage has been accomplished largely through the combined efforts of the EPI of WHO, UNICEF, the World Bank and the United Nations Development Programme, with support from non-governmental organizations, particularly the Rockefeller Foundation, Rotary International and the Save the Children Fund, which together co-ordinate their efforts through the Task Force for Child Survival. Behind the scenes scientists trying to develop new or improved vaccines scramble for support, most of it coming from the US National Institute for Allergy and Infectious Diseases or the Department of Defense, or from WHO's Special (extrabudgetary) Programmes for Research and Training in Tropical Diseases, Human Reproduction, or Vaccine Development. A decade ago, some foundations were generous contributors to research on diseases of the third world, but their interest seems more to have reflected fashion than a serious commitment to the problem. And one must add that, regrettably, few third world leaders have made health a high priority.

There has been only limited interest by the private sector in development of vaccines for the industrialized world, and virtually none in vaccines for the third world. 19/ The reasons are simple. Vaccines account for only 1 per cent of the profits of the pharmaceutical industry, and a greater percentage of their liability. One of the two new vaccines licensed in the past ten years is a subunit vaccine against Haemophilus influenza b, the main cause of meningitis and mental retardation in young children around the world. It consists simply of a bacterial carbohydrate conjugated to tetanus or diphtheria toxoids to augment the immunogenicity. The cost for each of the companies trying to develop that vaccine has been $8 12 million. The cost would almost certainly be higher for live vaccine vectors.

Another example: hepatitis-B virus is not only the cause of a serious infectious disease, but the principal known risk factor for hepatocellular carcinoma, which is particularly prevalent in Asia. Several vaccines are currently being produced, one of which is available to EPI for large-scale public health use at $1 per dose. Nevertheless it cannot be included in EPI because of cost. Because of the numbers of people involved, there must be incentives to develop and deliver

vaccines and essential drugs to the third world. It should at least be possible to establish cost-plus agreements with international agencies to develop potentially useful interventions, or joint ventures or affiliations in developing countries.

The imagination and resources of the private sector that were so instrumental in developing biotechnology should be engaged, and the private sector may find that it has something both to contribute and to gain. Failing that, developing countries that can afford it will have to rely on their own abilities, intellectual and material, for developing their own biotechnology. This is already happening in Cuba, India, Brazil and Mexico. It is important to note that several new vaccines now in field trials have, in fact, been developed by scientists in the third world - vaccines against leprosy (developed in Venezuela and India), leishmaniasis (Venezuela and Brazil) and dengue haemorrhagic fever (Thailand). Finally, third world countries have enormous foreign debt, much of which will clearly have to be written off. If even a small portion of that debt were restructured to be spent in local currency for health and education, several of the problems affecting the quality of life in the third world could be addressed.

The first world is a place where 13 per cent of the world's people consume the major part of the planet's crude resources, most of which come from the third world. In the United States we spend annually over $300 billion on "defence"; a single B-2 "stealth" bomber costs $532 million. In terms of personal consumption, $55 billion ($287 per adult) is spent annually on alcohol, $38 billion on tobacco and $22 billion on toys. 20/ We can afford to do more for health in the third world. Conscience should motivate us to do so, and self-interest supports the claims of conscience. In the first place, good public health translates into good economics. We save over $0.5 billion per year in no longer having to control smallpox (the global figure is $2.5 billion). Second, in our world there is nothing which is truly remote and no one from whom we are disconnected. AIDS has again demonstrated that: another example, dengue haemorrhagic fever, which has been ravaging the Caribbean, and for which an effective vaccine does not yet exist, is predicted to spread in epidemic proportions to parts of the United States. Finally, it is becoming more and more clear that poverty and disease have not only a moral impact but a political price. Ultimately, what is the cost of political turmoil? We have the resources to make vaccines and essential drugs available to the people of the third world; what we need are the imagination and the will.

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Notes

1/ State of the World's Children (UNICEF, Oxford, 1988).

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