OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

Genetic Engineering and Biotechnology Monitor

Issue No. 34 May 1991

N.B. This issue carries a special article on Vaccine potency and stability: trends in technological developments, by Drs. Carolyn D. Deal, Jitendra N. Verma and Bhupendra P. Doctor.

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

A. POLICY, NEWS AND OTHER EVENTS

UN and other organizations' news

Environmental resource management: educating the business leaders of tomorrow

Fifty leading academic institutions, industry and environmental experts from all over the world met at the European Institute of Business Administration (INSEAD) in Fontainebleau, France, on 11-12 October 1990, to discuss ways to develop sound environmental practices of the business leaders of tomorrow.

UNEP/IEO co-sponsored the two-day international conference in conjunction with the European Institute of Business Administration, the Management Institute for Environment and Business (MEB) and Tufts University, and in co-operation with the International Chamber of Commerce.

Among the issues discussed by the participants were the role of graduate business schools in environment management education and research; strategies for integrating the environment management curriculum into MBA and executive management programmes; the research priorities in environment and business; and future strategies for integrating environmental resource management into business school education and research.

Participants expressed a need for a network to exchange information on case studies and other educational material, and who to contact for further information. They provided an indication of priorities for a future research agenda.

A second meeting will be organized to involve business schools and universities in industrializing countries in this process of integrating environmental management in the education of future business leaders and government officials. (Source: UNEP News Release, 15 October 1990)

Priorities for UNEP/IEO

During the 1990s, the work of UNEP's Industry and Environment Office (UNEP/IEO) is to be concentrated in two key areas, following decisions made by UNEP's Governing Council in May 1989.

The first major goal is the establishment and operation of a network of industries and organizations dedicated to "Cleaner Production" - industrial production with less pollution and less waste. Cleaner production involves the development and application of low- and non-waste technologies as well as new management practices. It involves the dedication of industrial managers to improving their environmental record - and hence, often, their own productivity; and it involves hard thinking about minimizing use of scarce natural resources.

IEO's drive to develop a Cleaner Production Network, which will link interested parties and provide members with easy access to the latest information, starts with several industrial sector working groups, a newsletter, a computerized information service and a printed directory.

A year ago UNEP was able to co-ordinate the negotiations that led to the Basel Convention, designed to control the ways in which hazardous wastes are moved across national boundaries. A programme of training workshops designed to help countries manage these wastes has been set up by IEO along with the Cleaner Production network, which incorporates the aims of the Basel Convention but goes further. It emphasizes clean technologies, which will eventually minimize the need to dispose of hazardous wastes.

The second of IEO's aims is to prevent industrial accidents and to reduce their impact on the environment and human health when they do occur. To this end UNEP/IEO launched APELL - Awareness and Preparedness for Emergencies at Local Level - in 1988. APELL's purpose is to encourage local communities to develop emergency plans to deal with technological accidents. A handbook and newsletter have already been published, and the idea of APELL has been presented by IEO speakers to audiences all over the world.

Cleaner Production Network

As part of IEO's Cleaner Production programme, working groups were formed to assemble information on tanning, electroplating, textiles and halogenated solvents. Future groups will investigate government policies and the pulp and paper industry. The information gathered will be presented at a seminar to promote Cleaner Production and then incorporated into ICPIC (the International Cleaner Production Clearing House). To this end, a working group is studying how the data can be harmonized for computer storage and retrieval.

ICPIC, which is based on the EPA's Electronic Information Exchange System in the United States, underwent tests by trial users. ICPIC can be accessed by anyone with a microcomputer and a modem. It contains more than 400 case studies from all over the world, a calendar of events, a directory of experts, a bibliography and descriptions of international programmes on low- and non-waste technologies. It will undergo major expansion. (Source: UNEP/IEO Information)

FAO proposes "new" plan for Africa

Food production in Africa is in crisis. A few years ago the continent was 100 per cent self-sufficient; now it is only 80 per cent so. That food supplies are inadequate is well known. Less known is that the continent is also washing away. Studies from many African countries show that huge amounts of soil are being eroded in agriculture, removing far more nutrients than are ever added back through fertilizer use.

Now the United Nations Food and Agriculture Organization (FAO) claims it has a solution to the twin crisis: supplying large amounts of fertilizer as aid-in-kind to countries where the problems are most severe. Critics say the plan sounds like a rerun of mistakes made in the 1960s and 1970s when provision of fertilizers increased third world debt but did little to boost food production. The FAO denies that this is the case, arguing that it intends to use the chemicals only as a means of putting a brake on the downward spiral. After the initial step of providing fertilizer, FAO agents hope to get farmers' attention and persuade them to use new methods that will sustain agriculture over the long term.

By rapidly increasing food production - through use of fertilizers and other techniques - African farmers can be persuaded to stay with their fields and improve them, rather than move on as the land

becomes increasingly degraded. It is hoped the dual strategy will stop the continent-wide spiral of agricultural collapse. This plan, which FAO calls the International Scheme for the Conservation and Rehabilitation of African Lands, gets the thumbs-up from some soil scientists.

That erosion is taking a severe toll in Africa is indisputable. A recent FAO study of Zimbabwe concluded that each hectare (2.47 acres) of well-managed maize-growing land loses 10 tons of soil per year; small community farms lose 40 tons, and already degraded rangelands, up to 100 tons. The same pattern prevails throughout Sub-Saharan Africa. A study of 38 countries by the Winand Staring Centre for Integrated Land, Soil, and Water Research in the Netherlands showed that farmlands in all of those countries were losing far more nutrients through erosion than they could afford to apply as fertilizers.

In the past, aid organizations favoured a two-pronged approach to the joint crisis of erosion and low food production. They created massive, government-backed soil conservation schemes and provided large amounts of fertilizer. These tactics were temporarily effective, but failed in the long run, partly because farmers saw maintenance of dams and contour banks as a job for government, not themselves. With its new approach, FAO hopes to change attitudes and teach farmers that they can benefit personally by adopting techniques that conserve the soil.

The first step, supplying chemicals, should yield dramatic results. Africa currently uses very small amounts of fertilizer - 11 kilograms per hectare compared to 700 kilograms per hectare in the Netherlands. At the same time an average crop of grain removes 100 to 150 kilograms of soil. Studies gathered by FAO over 30 years in more than 50 African countries show that 1 kilogram of plant nutrients can produce an extra 10 kilograms of grain, 6 kilograms of oil crops, or 40 kilograms of roots and tubers.

At first, the FAO scheme may require large inputs of fertilizer - as much as 100 kilograms per hectare. That initial injection will provide a quick return in the form of crops that can be eaten or sold, as well as organic residues that can be returned to the soil for the next growing season. Recycling these residues will reduce the need for mineral input in subsequent seasons.

As it provides fertilizer, FAO will instruct farmers in efficient techniques for using it as well as instruction in other soil-conserving practices. They will also provide some other essential supplies, such as Rhizobium bacteria, which enable legumes to fix nitrogen. FAO has already helped several countries set up pilot plants for producing Rhizobium and shown farmers how to inoculate seeds with the bacteria.

FAO is also encouraging farmers to use new practices, such as planting grass strips along the contour lines to prevent rain run-off. A new FAO booklet shows trainees how to mark contours with simple equipment and plant grass strips of selected varieties. Such techniques have increased yields by 30 to 50 per cent in Ethiopia. (Extracted with permission from Science, Vol. 250, p. 740, 9 November 1990; Jeremy Cherfas. Copyright AAAS 1990.)

ICGEB news

Conference on genetically modified organisms for the 1990s

The Conference, to be held between 3 and 5 July 1991 at the ICGEB, Trieste, Italy, aims at being a forum for communication of the latest information on genetic engineering research, especially where this may soon lead to the production of organisms that could be released to the environment.

The topics will cover gene transfer and survival of micro-organisms in the environment; environmental effects on the expression of cloned genes in plants; Antisense RNA and fruit ripening; Viral and bacterial vectors for vaccine development; Baculoviruses as expression vectors; Antisense RNA in viral control and the transformation of plants, fish and animals.

Participation requirements: active involvement in research in recombinant DNA technologies, microbial genetics and ecology.

Closing date of applications - 31 May 1991.

For further information please contact Ms. Diana Viti, ICGEB, Padriciano 99, I-34012, Trieste, Italy. Tel.: (39-40) 3757333, Fax. (39-40) 226555.

Practical course on genetically modified organisms

The ICGEB (Trieste, Italy) is organizing a practical course on genetically modified organisms: safety in the laboratory and the environment, to be held from 1-3 July 1991, which is sponsored by the United Nations Environment Programme (UNEP).

The topics will cover:

Biological risk assessment;

Containment of GMOs in the field and the laboratory;

Monitoring methodologies for GMO field releases;

Comparative analysis of existing biosafety legislation;

Recommended procedures for safe practice;

Preferred host/vector systems;

Recombinants containing potentially oncogenic nucleic acid sequences;

Transgenic animals;

Databases and artificial intelligence in biosafety management.

The course is open to scientists from ICGEB member countries nominated through their National Scientific Focal Points and is limited to 30 students.

Participation requirements: basic knowledge of molecular biology and involvement in biosafety legislation, risk assessment and monitoring of field releases of GMOs.

Closing date of applications - 31 May 1991.

ICGEB will provide accommodation and local transportation. A small number of participants from non-ICGEB member countries will be supported by travel and subsistence grants sponsored by UNEP.

For further information, please contact Ms. Diana Viti, ICGEB, Padriciano 99, I-34012, Trieste, Italy. Tel.: (39-40) 3757333, Fax.: (39-40) 226555.

The course is sponsored by the United Nations Environment Programme (UNEP).

The virology group at ICGEB

Research activities are currently directed towards studies, at the molecular level, of two types of viral hepatitis: hepatitis B and enteric non-A non-B hepatitis.

The hepatitis B programme

Hepatitis B is a major world health problem with an estimated 200 million carriers of this disease world-wide. The predominant mode of transmission is parenteral, where chronic carriers constitute the reservoir for spread of infection to other susceptible individuals, either horizontally or vertically. Severe chronic hepatitis B frequently leads to premature death from liver failure. Chronic hepatitis B is also associated with the development of primary hepatocellular carcinoma (PHC) with risk of PHC development being about 300-fold that of age matched non-carriers.

The aetiological agent for this disease, hepatitis B virus (HBV), is a small (42 nm), partially double-stranded DNA virus. The host range of HBV is narrow, to date productive infections have been established only in human beings and higher primates. In permissive hosts viral antigens and DNA are found primarily within liver cells, which harbour abundant quantities of replicative and assembly intermediates as well as mature virions. The genome of HBV is circular DNA of only 3.2 kilobases in length which encodes at least four viral antigens: DNA polymerase (P), Core (HBc), Surface (HBsAg) and X (HBX) proteins.

The hepatitis B programme is focused on the following aspects:

1. Expression and characterization of functional domains of the X-protein (HBX).

2. Analysis of the enhancer element of HBV.

3. Novel approaches towards the design of a molecular vaccine for hepatitis B.

4. Lymphokine - derived immunostimulatory agents as potential adjuvants.

I. Hepatitis B virus X Protein: Of all the HBV-encoded proteins, X is the least understood. Recently, it has been shown to be capable of transactivating a number of viral and cellular promoters or enhancers. Most significant in this respect is transactivation of the long terminal repeat (HIV-LTR) of the human immuno-deficiency virus (HIV) by X, as it is the first evidence of an interaction between HBV and HIV at the molecular level. This supports clinical observations that a majority of AIDS patients also test positive for hepatitis B. Does HBV infection in any way predispose towards HIV infection? With the HBV X protein transactivating the HIV-LTR, it is possible that HBV infection may activate a latent form of HIV into full-blown AIDS.

The purpose is to understand the mechanism of this transactivation. A simple-minded mechanism would involve binding of X to its target DNA sequence(s), just like a number of known transcription factors. Preliminary evidence rules this out because X does not seem to bind DNA, and no consensus nucleotide sequence can be localized on target DNA. Transacting factors that do not bind DNA generally act by interacting with or modifying other DNA-binding proteins.

Scientists at ICGEB are tackling this problem by generating a number of site-directed mutants of the X protein. The transactivating properties of these mutants will define the functional domains of this protein. Simultaneously co-precipitation of X and other cellular proteins with anti-X antibodies is being pursued to define proteins capable of associating with X.

II. HBV Enhancer: Enhancers are cis-acting DNA elements that are able to potentiate transcription from RNA polymerase II (B) transcribed promoters independent of orientation and distance. They also confer tissue specific gene expression and most interestingly, they are often found transposed to proto-oncogenes thereby inducing tumour formation.

A major HBV enhancer has been mapped to a region between the surface and X genes. This enhancer can transcriptionally regulate at least three HBV promoters - the X and core promoters are located downstream while the surface antigen promoter is located upstream. It also binds several cellular proteins and exhibits liver specificity. A second liver specific enhancer has recently been identified adjacent to the core promoter of HBV. It may be developmentally regulated as excessive core gene expression is observed in advanced hepatocellular carcinoma when HBV replication is virtually absent.

The research programme includes a detailed mutational analysis of certain repeated sequence elements present within the major enhancer region of HBV. Cloning out enhancer binding factors from liver cDNA libraries using the "South-Western" technique is also under way. These studies are likely to help in understanding mechanism(s) of gene expression in cells infected with the virus, its role in pathogenesis of hepatitis and hepatocellular carcinoma and tracing the evolutionary origin of hepadnaviruses.

III. A Molecular Vaccine for Hepatitis B: The synthetic peptide methodology has proved immensely useful in mapping important domains within surface antigen proteins of a variety of pathogens. However, keeping the phenomena of MHC-restriction and antigenic variation in mind, it appears unlikely to the ICGEB researchers that a synthetic peptide vaccine will prove effective in a genetically outbred human population. To overcome these problems a novel approach has been adopted, where proteins will be designed that code exclusively for a variety of selected immunologically and functionally relevant determinants of the hepatitis B surface antigen. The synthesis, assembly and expression of the gene for one such construct is currently under way. It is anticipated

that studies of this nature will also help elucidate some basic principles applicable towards the design of molecular vaccines in general and in addition provide an excellent model system to study the mechanism of antigen processing and presentation.

As a corollary to these above-described studies, the researchers are using synthetic peptides extensively to identify important and potentially useful regions of HBsAg. Recent efforts have also been focused at reconstructing conformation-dependent antigenic determinants of HBsAg with synthetic peptides.

The development of a recombinant HBV vaccine is also planned. This will include sequences from the pre-S regions to improve the immunogenicity as compared to the vaccines currently available. Certain novel approaches aimed at maximizing HBsAg expression in cultured cells are currently being pursued to provide a cost-effective alternative to the presently available recombinant HBV vaccines.

IV. Lymphokine-derived - Immunostimulatory Agents: Broadly speaking, the approach here involves the use of synthetic peptides from lymphokine sequences that are capable of potentiating the immune response against a given immunogen. Our initial efforts are focused on human interleukin-1B (IL-1B) since a non-a peptide derived from this protein with immunostimulatory activity has already been described. We have shown that coupling of this non-a peptide sequence to a given peptide immunogen can confer in-built adjuvanticity at least in the murine system. The researchers are currently examining the potency of other IL-1 derived sequences and, in particular, peptides that combine various functional regions of the native IL-1 protein. It is expected that such "bonsai" versions of IL-1 will find application as adjuvants in a variety of vaccine preparations including that for hepatitis B.

By enhancing immunogenicity of a given vaccine in such a manner, it should be possible to reduce the number of immunizations required to confer total protection. This would then make it more amenable to global immunization both by reducing cost and increasing efficacy in terms of lower drop-out rates and thereby be of particular benefit to the developing world.

The enteric Non-A, Non-B hepatitis programme

The development of diagnostic tests for viral hepatitis A and B has led to the realization that another form of viral hepatitis exists. The diagnosis of this Non-A, Non-B (NANB) hepatitis is currently one of exclusion. Two major forms of NANB hepatitis are recognized world-wide.

Of these, enteric NANB hepatitis follows a faecal-oral transmission, with contaminated drinking water as the major source of infection. This form of hepatitis is endemic to the Indian subcontinent and a number of major epidemics have been reported in the last few years. A sero-epidemiologically similar disease has also been reported from South-East and Central Asia, parts of Africa, the Middle East, the Soviet Union, as well as part of North and Central America. Current estimates project that with the availability of definitive diagnostic protocols, this form of hepatiitis may exceed hepatitis B as the world's most common form of hepatitis.

The candidate aetiological agent has been identified as a 27-34 nm viral particle in the stools of patients. The ICGEB effort into enteric NANB hepatitis is aimed at initially cloning the genome of this virus. To this end, the Centre has set up an animal model system by transmitting the disease from human patients to rhesus monkeys by inoculation of infectious stool suspensions. The researchers are currently preparing subtractive cDNA libraries from animal materials in bacteriophage lambda. These libraries will be screened with convalescent sera to identify specific clones. Regions of the viral genome once cloned will provide the basis for developing definitive diagnostic reagents, and later on, a recombinant vaccine. Based on minimal nucleotide sequence information available, polymerase chain reaction (PCR) based strategies are also being pursued to develop a confirmatory diagnostic test for enteric NANB hepatitis.

The Virology Research Group at ICGEB is well equipped with modern day facilities and instrumentation requisite for competitive research. It also has ready access to a protein sequencer, automated peptide and oligonucleotide synthesizers and an animal house.

Biotechnology of mining - bacterial leaching of minerals in Chile

Chile, like many other developing countries, is highly dependent on mining. Copper exports account for close to 50 per cent of the foreign currency that the country receives annually. It is not surprising, therefore, that one of the priority areas of the National Committee for Biotechnology of Chile is the bacterial leaching of minerals.

As the grade of the existing copper ores becomes lower and lower through continuous exploitation, bacterial leaching and hydrometallurgy become more important in the mining business. Some experts have estimated that 20 per cent of all the copper presently extracted in the USA is produced through processes involving bacterial leaching. The commercial feasibility of the bacterial leaching process depends a lot on the type of copper ore involved.

High grade sulphide copper ores (above 0.5 per cent) are extracted through the classical pyrolytic process, which is faster and more economical but which has serious problems due to atmospheric contamination. Oxide ores of copper can be chemically leached with concentrated sulphuric acid treatment of the minerals in heaps or piles. The most abundant copper ores, however, are chalcopyrites, which are mixed cuprous and ferrous sulphides. These sulphide ores are quite recalcitrant to chemical leaching and can only be leached effectively through the action of bacteria. The bacteria that can carry out this process are very peculiar micro-organisms, since they require a very acid environment (below pH3) to grow and derive their energy from ion oxidations. There are a number of micro-organisms that can participate in the leaching process, including bacteria from the genders Thiobacilli, Leptobacilli and Sulpholobus. However, the best known and most abundantly found is Thiobacillus ferro-oxidans. This gram negative bacterium can fix atmospheric CO_2 and N_2 to obtain carbon and nitrogen compounds for its metabolism and uses atmospheric O_2 to oxidize ferrous to ferric ions and sulphides to

sulphate. These oxidations are its sources of energy.

T. ferro-oxidans can attack chalcopyrite directly to solubilize ferric and sulphate ions. The ferric ion can, in turn, oxidize the cuprous ion of the chalcopyrite to cupric ions that go into solution.

Empirically the action of these bacteria can be observed in a situation when a heap (pile) of ore containing copper oxides and chalcopyrite is irrigated with dilute sulphuric acid.

Analysis of the effluents emerging from such a heap (pile) reveals that there is an initial yield of solubilized copper due to the fraction of copper oxides present in that ore. After a variable period of several weeks, during which the heap (pile) has been intermitently irrigated with the acid solution, the effluent again contains soluble copper in appreciable amounts and also shows the presence of leaching bacteria in numbers that normally range from 10^5–10^6 bacteria/ml. The extraction of copper by this method is very slow, since it may take several months to achieve a commercially significant percentage of the copper sulphide in the ore. Very often, however, the low grade ore that can be treated in this way has already been withdrawn from the mine together with richer ores and dumped due to the process. Copper recovery from these ores, therefore, constitutes a welcome bonus for the mining operation.

How can biotechnology help such a process? The reply of the mining engineers and executives is to ask the bio-scientists to try and accelerate the process. From laboratory experiments performed with columns packed with typical ores, it can be demonstrated that growth of the bacteria in the ore is intimately related to the yield of copper leached from the mineral. However, there are a large number of other factors such as the granulometry of the ore being treated, the fluid dynamics within the heap of ore, the composition of the ore, the temperature outside and inside the pile, the partial pressure of the atmospheric gases required by the bacteria, etc. For this reason, a biotechnology project that deals with improving the bacterial leaching of copper ores requires a large transdisciplinary team of researchers that ranges from basic bacteriologists and molecular biologists to mining engineers, geologists and electrochemists.

In 1985, a group from the Chilean National Committee for Biotechnology, won the approval of the Chilean Government, the United Nations Development Programme (UNDP) and the United Nations Industrial Development Organization (UNIDO) for a project to do research in the bacterial leaching of Chilean copper ores. This project had a first stage covering 1985–1987 and second stage 1988–1990. It has involved six institutions: The University of Valparaiso, the Technological Institute of Chile, the Centre for Mining and Metallurgy and the Chilean Copper Corporation (CODELCO). CODELCO is one of the largest copper mining companies in the world since it controls all the State-owned mines of Chile. There are more than 50 researchers that have actively participated in this project, which has had a direct budget of approximately US$ 2 million and much larger resources additionally, if in-kind contributions are considered. This major project has been divided into three subprojects dealing with the biological, the engineering and the mining aspects. Table 1 shows some of the research topics that have been investigated by these three different groups.

Table 1

Scientific research activities of the three subprojects of the bacterial leaching of minerals

Biological subproject	Engineering subproject	Mining operations subproject
Study of the T. ferro-oxidans proteins and enzymes involved in the ferrous ion oxidation.	Computer models on the leaching process in experimental models.	Pilot mineral leaching pile for demonstration of the process for potential users.
Biochemistry and genetics of CO_2 fixation in these bacteria.	Ideal conditions for the leaching of concentrates in shaker tanks.	Monitoring of various parameters including flora of micro-organisms in actual mining operation that involve leaching.
Chemotaxis of bacterial attachment to minerals.	Monitoring of CO_2, O_2, pH and other parameters under experimental conditions.	Design of piles and dumps for bio-hydrometallurgy.
Strain identification by DNA probes and specific antibodies.	Fluid dynamics of different kinds of piles and heaps.	
Genetic manipulation of Thiobacilli.	Design of inoculating methodology.	
Selection of resistant strains to toxic metals.		
Lipopolysaccharides and proteins involved in attachment of bacteria to mineral surfaces.		

After five years, considerable progress has been achieved in understanding the biochemistry and physiology of the bacteria, in determining some of the key parameters that limit leaching in heaps and piles, in designing the bio-leaching operations and in monitoring the progress of a leaching process. From a level of practically zero knowledge about the process, Chilean researchers have constituted a group with expertise that is recognized internationally. A large number of publications and two patent applications have resulted from the work carried out. More important, however, a large number of young researchers have been trained in advanced biotechnology techniques and have been infused with the philosophy of working in topics of high relevance to the country and of participating in large interdisciplinary teams.

Although the UNDP sponsored project came to an end in December of 1990, the researchers involved in this project will continue to work in this area with funds from the Chilean National Fund for Science and Technology and from international sources. Prof. Carlos Jerez of the Department of Biochemistry of the Faculty of Medicine of the University of Chile has just won an ICGEB Collaborative Research Project to work on "Studies of the stress response in biomining microorganisms. Possible implications in the improvement of bioleaching process", which he will carry out in collaboration with the laboratory of Dr. Hector Torres of Argentina.

Mining biotechnology is still in its infancy and certainly much work has yet to be done to achieve large breakthroughs in increased productivity. However, this is clearly an area in which the developing countries must keep alert and which can be used to establish research teams and to link the scientists to the problems of their societies.

Regulation

EC biotechnology laws are confusing, says CEFIC

Industry representatives have criticised the plethora of European directives concerning biotechnology research as being "confusing and often conflicting and in danger of vastly over-regulating biotechnology and inhibiting its development in Europe".

In a paper presented at the Brighton Crop Protection Conference, the European Chemical Industry Federation's Senior Advisory Group on Biotechnology (SAGB) said, "European countries and the European Community are wrestling with a problem complicated by the introduction of concepts such as environmental harm, whether the technique or the organism should be regulated and the relationship to existing legislation".

The founding and board members of the SAGB, which was set up in 1989, are Ferruzzi, Hoechst, ICI, Monsanto Europe, Rhône-Poulenc, Sandoz and Unilever.

The SAGB levelled particular criticism at the Council directive on the deliberate release of genetically modified organisms to the environment, which has been adopted this year. The directive calls for a case-by-case notification and endorsement procedure by a competent national authority.

The directive distinguishes between research and development projects and finished products, for which companies will also have to consult the European Commission and other Member States.

The SAGB said the directive will involve duplication of testing and data review processes for products as there is considerable overlap with existing product approval mechanisms. The confusion created by this directive is likely to adversely affect competitiveness and jobs, the organization said.

Also, the directive distinguishes products according to the technology used, such as recombinant DNA, micro-injection, micro-encapsulation, nuclear and organal transplantation or genetic manipulation of viruses.

The SAGB says the correct categorization should be by product application, such as pesticide, food and pharmaceutical, which would be a way of discriminating on the basis of safety criteria.

Another, the Council directive on the contained use of genetically modified organisms, was also adopted in 1990. Other proposed directives that will affect biotechnology research include directives on pesticides, new foods, protection of workers, intellectual property protection, plant breeders' rights, marketing transgenic animals, food labelling, harmonization of food additives and productivity.

Biotechnology is a priority area that has been newly introduced to the EC's fourth action programme spanning 1987 to 1992. (Source: European Chemical News, 26 November 1990)

German regulatory mechanisms

East and West Germany may have been unified, but the picture for the regulation and approval of biotechnological production and products is far from uniform. Indeed, by occupying centre stage in the thoughts and actions of German politicians and administrators, reunification may slow progress towards a truly integrated and coherent framework for the development of recombinant DNA products.

The Gene Law enacted in July 1990 has eased the way for companies to obtain approval for production processes. Generally, most systems envisaged for production will fall under the lowest safety level defined by the Law; applications will not, therefore, need to come before a public hearing (as was the case under the Federal Emissions Act). The mere existence of the Gene Law has removed the legal objection to Hoechst's (Frankfurt) production system for recombinant insulin.

Hoechst is expected to submit a registration application for recombinant insulin to the BGA in 1991 and the agency's position, enshrined in German pharmaceutical law, remains that the compound is completely novel. A full three-year trial to establish quality, safety, and efficacy will, therefore, be required.

If one effect of the Gene Law is to focus objectors' attentions on medicinal approval, the other is to devolve responsibility for process approval onto individual German states, which now number 16. The Law stipulates that the Länder authorities must submit applications and supporting data to the Central Biological Safety Committee (ZKBS) in Berlin, who advise on the appropriate safety classification of the proposed project. That classification prescribes the subsequent administration of the project, notably whether a public hearing is necessary. The ZKBS advice is not binding, so that in theory at least, a second Länder-based committee could reclassify projects.

Nevertheless, there is clear political pressure in some of the German states to decentralize this critical decision-making state. While industry and the Federal authorities may be sceptical about the ability and utility of state-based committees, the Länder governments themselves are clearly determined to have as much say as possible in applying the Gene Law. (Extracted from Bio/Technology, Vol. 8, November 1991)

General

Convention breaks down over protecting gene pool

A global convention to conserve biological diversity could fall apart if developing and industrialized countries cannot settle a dispute over who should have access to biotechnology.

The future of the convention was thrown into doubt when developing countries, led by Brazil, India and China, demanded that the convention must allow them access to expertise in biotechnology that would enable them to exploit their biological resources. Industrialized countries balked at the proposal, insisting that the convention should concentrate on conserving areas of great biodiversity that are not protected by existing conventions and agreements, such as the Ramsar Convention on Wetlands and CITES, which controls international trade in endangered species.

For conservationists, the convention is much more than icing. They worry that the North-South dispute will delay the only convention that could help to stem the massive destruction of biodiversity. Simon Lyster, of the World Wide Fund for Nature, said that while both lobby groups and the developed countries believe the convention should include technology transfer where it is appropriate to biodiversity, the developing countries "will not get by 1992 a convention that gives them access to multi-million dollar technologies. The industrialized countries will not give away their secrets by 1992. If the developing countries push too hard, for too much, too soon, we may not get a convention at all".

Agriculture and industry depend heavily on the world's biodiversity. According to one study by American writer on conservation, Robert Prescott-Allen, genes from wild plants a d animals contribute almost five per cent of the US's gross domestic product. Most of the world's biodiversity exists in developing countries. (Source: New Scientist, 15 December 1990)

European industry pools resources for common views

With "Open European Frontiers" only one year away, Europe is seeing many transnational activities to pool resources and remove hurdles that could obstruct the formation of one large European market. These take place at governmental and industrial levels.

A reflection of this intention is the recent (22 November 1990) establishment of an **Animal Cell Technology Industrial Platform (ACTIP)** by representative European industries involved in animal cell technology.

The initiative to establish an Animal Cell Technology Industrial Platform finds its origin in the Commission of the European Communities in relation to the CAN-BRIDGE T-Project (T stands for "targeted") on animal cell technology. The BRIDGE programme, which runs from 1990 to 1993, is promoting European collaboration in the field of research and development to provide basic know-how and a competitive advantage for European industry. In order to allow industry an opportunity to present commonly held views about the direction of future research and an opportunity to be timely informed on results, the Commission strongly supports the elaboration of a platform of industries having an interest in this T-project, but do not wish to participate in contractual research.

ACTIP's objectives are:

1. The development of a common attitude to provide an industry perspective to publicly supported R&D in Europe regarding methodologies and technologies for animal cell culture.

2. The identification and development of solutions to problems and obstacles that may arise while implementing new methodologies and technologies of animal cell culture and the commercialization of resulting products.

3. Informing the public so that it may recognize the positive contributions biotechnology makes through animal cell culture; and monitoring of public opinion in this regard.

ACTIP aims to develop into a truly pan-European cross-section of industry. Therefore, industries wishing to participate in ACTIP are invited to contact the secretariat. ACTIP in particular welcomes the participation of industries located in southern European countries. Also industries located in EFTA countries and former Eastern European countries are invited to pool their resources in ACTIP.

For more information:

ACTIP Secretariat
c/o H&M Consultancy b.v.
Dr. Helma Hermans
Scheveningseweg 9
2517 KS THE HAGUE
The Netherlands
Tel.: (31) 70 356 07 53
Fax: (31) 70 361 46 95

(Source: ACTIP News Release, 19 December 1990)

Biotechnology and biodiversity

1992 will see a major United Nations conference in Brazil, marking the twentieth anniversary of the Stockholm Conference, which first put environmental issues on the international political agenda. One theme that the conference, the United Nations Conference on Environment and Development (UNCED), will address, is that of "biodiversity". Meanwhile, the International Federation of Institutes for Advanced Study (IFIAS) and the African Centre for Technology Studies (ACTS) are planning the third international symposium of the IFIAS International Diffusion of Biotechnology Programme. The symposium will be held in March 1991 in Nairobi, Kenya. It will be organized by ACTS in conjunction with the Biodiversity Conservation Strategy programme of the World Resources Institute (WRI), the International Union for Conservation of Nature and Natural

Resources (IUCN) and the United Nations Environment Programme (UNEP). Awareness of the role of genetic resources in biotechnology research has led to debates on the way the benefits of biotechnology are shared among the industrialized and developing countries. The issue of ownership is compounded by the alarming rate at which biodiversity is being lost worldwide. The symposium will explore and identify incentives and measures which can promote technological innovation in biotechnology, while guaranteeing the "equitable utilization" of biodiversity. The ideas generated will contribute to current efforts to formulate a global convention on biodiversity.

Details from: John Mugabe, IFIAS-Maastricht, Witmakerstraat 10, 6211 JB, Maastricht, The Netherlands or on +31 43 250465. Fax: +31 43 218820.

Ecologically oriented growth for the third world

When environmental issues were on the political fringe in the industrialized world, developing countries often considered them an unaffordable luxury.

Now there is a growing consensus in rich and poor nations alike that conservation and sustainable development are inextricably linked. Traditional ecological concerns such as business regulation and wilderness protection have been recast in a new, human context.

The upcoming United Nations Conference on Environment and Development, set for June 1992 in Brazil, will reinforce the linkage. Meanwhile, European, third world and international organizations are striving to build North-South partnerships for ecological and humane growth.

Joseph Wheeler, chairman of the Paris-based Organization for Economic Co-operation and Development (OECD) Development Assistance Committee, says rapid global population growth makes the link imperative. Poverty is often a cause and a result of environmental degradation because the poor are forced to farm marginal lands, to cut forests for fuel, to exploit nature in order to survive. Mr. Wheeler believes that "an accelerated effort to reduce poverty, including high priority to family planning, should be the first line of attack" on environmental issues in developing countries.

The United Nations takes up developing-world ecological issues through several agencies. The Nairobi-based United Nations Environment Programme is considered to be "the environmental conscience of the UN system". UNESCO in Paris manages programmes to protect more than 300 World Heritage sites and nearly 300 biosphere reserves.

Among other groups with environment-oriented projects, the International Chamber of Commerce supports various ecological endeavours and the World Bank has helped countries set environmental strategies, and in 1990 made environment-oriented loans to Brazil, the Côte d'Ivoire, Madagascar and Poland. The International Institute for Environment and Development in London researches land use, sustainable agriculture, environmental economics and climatic change, while Oxfam of Oxford, UK, organizes such diverse efforts as regreening in Indonesia, water harvesting in Burkina Faso and development initiatives in the Amazon region.

Environmental issues in the developing world are often interwoven with politics. Agencies find that land reform, democratization, community empowerment and greater justice for women are essential parts of ecological improvements. At the same time, the environment in developing countries is affected by the practices of multinational companies and the imbalances of the international economic order.

The Greens Party in Germany is among the European organizations concerned with the environmental impact of business and aid on the developing world.

The World Wide Fund for Nature (formerly World Wildlife Fund) has made businesses environmental partners through debt-nature swaps. With their help, WWF has purchased some of the debt of Costa Rica, Ecuador, the Philippines, Madagascar and Zambia, which have agreed to apply the equivalent in their currencies to local conservation.

The International Union for Conservation of Nature and Natural Resources (IYCN), based in Switzerland, works closely with WWF, the UN, the World Bank and others.
(Source: International Herald-Tribune, 12 November 1990, Advertising Section, article by Barbara Borst)

Africa needs science, not just technology

Aid agencies are hindering efforts to combat starvation in Africa by refusing to fund basic science, the president of the African Academy of Sciences said in London this week. Thomas Odhiambo told the SOS Sahel Agency that advanced research such as molecular biology was needed to solve the problems faced by farmers and herders.

Odhiambo laid some of the blame for weak science at Africa's own door. Its universities are "in tatters", he said. Those that were thriving 20 years ago are now in decline. Libraries are 10 years out of date.

But he also argued that Western donors ignore the long-term research in medicine, forestry and agriculture that is needed for economic development. Such research must concentrate on species of pathogens, crops and livestock native to Africa.

There are big gaps even in the basic knowledge of key ecosystems. For instance, little is known of the tropical grasses on which livestock depends. In droughts, farmers were blamed for overgrazing. But it turned out that termites often did the damage by eating grass roots during dry periods instead of their usual woody foods.

Odhiambo said pressure from agencies to produce quick results stunted science. "They always want results in three years", he said. "But you have to gather a great deal of background knowledge first".

He held up as a lone success the 40-year Onchocerciasis Research and Control Project to eliminate river blindness. Funded from the early 1970s by three United Nations agencies, it is planned to run for another 20 years. Odhiambo said the project had succeeded in developing new technologies for controlling the spread of the worm that carries the disease, new ways of producing a vaccine and new ways to treat infection. As a result, river blindness, which previously affected

as many as 80 per cent in some villages, is now much less common.

Biotechnology centres are planned in Kenya and Nigeria. They could produce new varieties of crops in three years rather than the 15 it takes by conventional breeding. These should be attractive to donors because they would weaken dependency on strains of crops that have high yields but are not robust enough for African conditions.

"It will be science transfer, not technology transfer", said Odhiambo. Molecular biology would be relatively easy to transfer because it uses cook-book techniques that are easy to adapt to Africa's particular problems.

Odhiambo said universities have got so bogged down in teaching undergraduates that they fail to do research. Programmes for post-graduates are weak and for post-doctoral students non-existent. Without them, effective research is impossible.

Good researchers are held back by their isolation, said Odhiambo. Many African countries are too small to support much scientific research and the continent needs a unified scientific community. (Source: New Scientist, 17 November 1990)

AIDS vaccine tests "more urgent" than HIV research

Trials to test whether potential AIDS vaccines can prevent disease should begin as soon as possible, without waiting to find out precisely how they work. This is now the official policy of the National Institutes of Health in the US, which considers that the need to stem the world-wide AIDS epidemic is greater than the need to do basic research on HIV.

Wayne Koff, head of vaccines research at the NIH, outlined the policy to a meeting of AIDS researchers in Paris in November 1990.

One option is to continue with basic research using animal models, before testing the effectiveness of a vaccine on people, said Koff. Basic research should aim to pinpoint the component of HIV that makes it pathogenic, establish the range of genetic variation between strains of the virus, and understand how infection crosses the mucous membranes.

Alternatively, he added, we can accept that we already know far more about HIV than many other viruses and adopt the "applied research" strategy. This means expanding existing safety trials in people while continuing and intensifying animal research so that "phase II trials", designed to find out how effective a vaccine is, can follow "as soon as possible".

Koff believes it is unrealistic to expect any vaccine to prevent the initial infection with HIV. Instead, researchers should aim at a vaccine that prevents HIV from becoming established in the body. Scientists must also continue work on immune therapies, inoculations that will boost the response of the immune system in people who are already infected with the virus.

The past year has seen several animal experiments that provide solid evidence that vaccines can protect against infection with simian immune deficiency viruses. In addition, safety trials at Johns Hopkins University and elsewhere in the US have shown that several potential vaccines are safe in healthy volunteers and that they stimulate antibodies and cells of the immune system.

However, there remain vital questions about how long this protection lasts in animals. And researchers still do not know exactly what part of the immune response is important in resisting infection with HIV.

Trials would need to be in areas where a relatively high proportion of the population is infected to keep the sample size manageable. For example, where 3.5 per cent of the population is infected, a trial could include as few as 450 volunteers if the vaccine worked 90 per cent of the time. If the vaccine worked only 60 per cent of the time, however, the sample population would need to be larger than 2,000. And if only 0.2 per cent of a population were infected, researchers might need to try the vaccine on as many as 40,000 volunteers.

Such trials raise major ethical problems. For example, some countries with a high incidence of infection do not have the medical infrastructure to conduct properly monitored trials. Restricting trials to people whose behaviour puts them at high risk of HIV infection, such as intravenous drug users, may be politically questionable.

It would be unethical not to warn people receiving trial vaccines against high-risk activities. According to Koff, researchers would have to assume that advising people against risky behaviour would decrease the incidence of infection in the trial group by around 30 per cent. The group would have to be enlarged to allow for this.

Most scientists, both in the West and the third world, now accept that there is no choice about moving quickly into human trials. (Source: New Scientist, 10 November 1990)

Third world AIDS programme short on funds

AIDS research in poor countries faces large cuts in 1991 because of a shortfall in the World Health Organization's funds. The rich nations that pay for WHO's Global Programme on AIDS have so far provided only £35 million of the £50 million set for the next budget.

The budget for 1991 had planned only a modest increase of about 11 per cent over last year's figure, even though the WHO has revised its projections of the scale of the pandemic sharply upwards. Officials say that up to 30 million people may be HIV-positive by the end of the decade, most of them in the poorer countries

The programme's biggest donors over the past three years have been the US, Sweden and the UK, who have given £32 million, £22 million and £14 million respectively, as "unspecified funds" - money for WHO to allocate to countries as it sees fit. By contrast, Germany has given only £465,000 of unspecified funds in this period.

Some countries, including Germany, prefer to earmark their donations for specific countries or

projects. However, the total of earmarked funds the programme receives in a year is only about £12.5 million and highly variable. Although it welcomes any donations, WHO prefers unspecified grants. (Source: New Scientist, 8 December 1990)

ATCC releases two polyclonal antisera and offers new Saccharomyces cerevisiae strains

The American Type Culture Collection (ATCC) has announced the release of two important polyclonal antisera: Barley Yellow Dwarf Virus Antiserum, RPV serotype (BYDV-RPV), ATCC Catalogue No. PVAS 524; and Tomato Spotted Wilt Virus (lettuce isolate) Antiserum, ATCC Catalogue No. PVAS 450. BYDV is one of the world's most widespread cereal crop viruses and of major economic concern. TSWV is of equal concern to vegetable and ornamental crop growers. Such high-quality antisera will be valuable to diagnosticians and researchers for use in serological assays to detect the presence of these two viruses.

ATCC has over 850 overlapping genomic DNA clones isolated from Saccharomyces cerevisiae available for immediate distribution. The cloned sequences were identified by M. Olson. The clones, in bacteriophage lambda vectors, have an average characterized insert site of 15.6 kb.

The clones are supplied as frozen aliquots of bacteriophage lysate. They are available to qualified researchers at a price of $45 for non-profit and $70 for commercial and non-US organizations. Written orders are required from first-time customers.

Details from: ATCC Sales, 12301 Parklawn Drive, Rockville, MD 20852, USA or on +1(301) 231-5524. Fax: +1(301) 231-5826. (Source: ABA Bulletin, Vol. 5, No. 5, October 1990 and Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Genome database goes on-line

The Genome Data Base (GDB) has gone on-line as the one computer data base designated by the Human Gene Mapping Workshop executive committee as the repository for all human gene-mapping information. GDB is a collaborative project involving Howard Hughes Medical Institute and Johns Hopkins University School of Medicine. It was tested successfully at the Human Gene Mapping Workshop in Oxford, England, in September 1990. Scientists can use GDB free, at least initially, to access gene-mapping information on-line in the US or abroad, via Telenet or Internet. Besides its use for gene-mapping information, GDB will also function as the repository for medical information on human genetic diseases and will encompass Victor McKusick's "Online Mendelian Inheritance in Man", which contains information about more than 5,000 inherited traits and diseases. GDB is built on the Sybase relational data base system and uses a Sun hardware platform, but it is designed to work in a network environment with any personal computer and multiple terminals. New registrants can call GDB's headquarters at Johns Hopkins' William H. Welch Medical Library to receive a free communications package, user manual, and password. The telephone number is (301) 955-7058. (Reprinted with permission from Chemical and Engineering News, p. 33, 5 November 1990. Copyright (1990) American Chemical Society)

Gene machine could speed up human genome project

Chemists at the University of Wisconsin have developed a prototype machine for unravelling the sequence of genetic material that runs 25 times as fast as machines now in use. If the team can construct a successful commercial device, this should cut the cost of the human genome project and speed it up.

The research team that built the new device was led by Lloyd Smith, a chemistry professor who created the first automated gene sequencer five years ago. Smith's new device, which uses thin capillaries filled with gel, rather than the present slabs of gel, could be available commercially within three years.

Gene sequencing is a three-stage process. First, scientists break the DNA down into short fragments using enzymes. They then label these fragments with fluorescent tags. A mixture of these fragments, each terminating at a particular base is applied at one end of a slab of acrylamide gel. The scientsts apply an electric voltage to the gel, and the different fragments move in response to the voltage. The fragments travel at different rates according to their size. In this way they separate into distinct bands.

A laser beam shining through the gel can read off the sequence of these bands and feed this information into a computer. From this data, the computer calculates the sequence of the original DNA.

But Smith's advance affects only one stage of sequencing, and the others will have to catch up if the human genome project is to benefit.

Smith and his colleagues reported their work in two journal articles: in Nucleic Acids Research (Vol. 18, p. 4417) and Analytical Chemistry (Vol. 62, p. 900). He will soon apply for a patent on further developments of the technique, and a University of Wisconsin agency will license the invention to certain manufacturers.

One company involved may be Applied Biosystems, which already produces automatic sequencers. Smith works as a consultant for the company. The other three firms now in the sequencer market, Du Pont, Hitachi and Pharmacia, may also be interested in the technology. (Extracted from New Scientist, 27 October 1990)

France boosts human genome research

Just two weeks after the official start of the massive US programme to sequence the human genome, France has decided to boost its own research efforts with the aim of efficiently gathering many of the fruits of research on the human genome. To speed up the process, a four-year Anglo-French Eureka pre-competitive technology research project, Labimap 2001, aims to develop and market a series of machines to automate time-consuming sequencing operations.

After consultation with leading French researchers, the Ministry of Research and Technology has decided to set up a new public body with its own scientific advisory committee, to distribute an extra FF 50 million next year and FF 100 million in 1992. The grants will go to some of the

500 researchers and 67 laboratories already engaged on genome research. The main role of the new body is to co-ordinate the national effort, ensure international liaison and encourage the commercial exploitation of discoveries.

France has already played a key part in human genome research, through its Centre for the Study of Human Polymorphisms (CEPH). With a FF 40 million annual budget (mostly State-funded but also supplemented with grants from the US National Institutes of Healt.., ..e Howard Hughes Medical Institute and national charities), CEPH makes available to researchers throughout the world a unique bank of genetic material from over 60 very large families. The aim is to use standard material more efficiently to localize and sequence the genes responsible for the 4,000 known hereditary illnesses. (Extracted from Nature, Vol. 347, 25 October 1990)

Genome project to tackle mass screening

Planners on the Human Genome Project have tentatively agreed to take the lead in a pilot project to screen the public for cystic fibrosis (CF) in what will be the first such project in the United States. At a meeting of the project's advisory committee, panel members advised project director James Watson to start looking for government and private partners in a pilot screening project.

Several of the National Institutes of Health (NIH) have expressed interest, as have other branches of the US Government, including the Agency for Health Care Policy Research, a branch of the Public Health Service.

Scientists have been working to map and sequence the human DNA molecule for two years while widespread testing for genetic diseases - a major application of the research - has languished. But before widespread testing can begin, policy-makers must tackle legal and ethical issues that have prevented the setting up of any genetic screening trials in the United States while trials have gone ahead elsewhere.

Unlike countries such as the United Kingdom and Denmark, which have already begun testing, the United States has no public health programme. The CF test is expensive - about $300 per sample - beyond the pocket of many potential subjects.

With mass testing and greater co-ordination, those costs could drop. Fewer than 3,000 CF tests are at present processed each year in the United States. In contrast, Britain, where laboratories this year will process some 50,000 samples and where public health care pays for much of the education, counselling and follow-up service, the tests cost only $2 each.

US policy-makers must also overcome a shortage of testing laboratories and genetic counsellors. A draft report from the genome project's working group on ethics, legal and social issues points out that "there are 3.5-4 million births in the United States each year. If even a small fraction of those requested CF testing, the system would be overwhelmed".

These logistical problems are overshadowed by the abortion issue: critics claim that testing is simply a rationale for abortion, leading some genetic disease organizations to shy away from screening as one controversy they can do without.

The debate about CF is further complicated by the disease's complex genetic origin. The defective gene, discovered last year, is found in only some three quarters of those who carry a genetic weakness for CF. Various mutations of the gene account for the rest. At best, current US tests can identify only 84 per cent of the carriers. Some groups have called for a moratorium on testing until a 95 per cent detection rate is reached, but without a research breakthrough, the 90 per cent rate now achieved by one Danish group may be the practical limit.

Deciding which of these issues are serious hurdles and which have simple solutions must wait for a large-scale pilot project. But some genome project planners are concerned that becoming involved in genetic screening could shift the emphasis of the effort from basic research to public policy - and delay the completion of the genome map.

Breast cancer and diabetes now seem to have genetic links, and a workable test would no doubt spur calls for widespread screening for them as well. (Source: Nature, Vol. 348, 13 December 1990)

First European experiment in human gene therapy

A team of Italian researchers is expected to receive permission for the first human gene therapy experiment in Europe before the end of the year, setting the stage for further experiments in other countries.

Claudio Bordignon of the Istituto Scientifico San Raffaele in Milan is the leader of the team and was an associate of the group that in July 1990 won approval for a similar experiment from the Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH) (see Nature 346, 402; 1990). Bordignon says that there are groups in the Netherlands and France that will soon be ready to perform gene therapy experiments.

Bordignon expects speedy approval for the experiment because his protocol is more conservative than that approved by RAC in the United States. He is treating a three-year-old child for a rare inherited immune disorder caused by a deficiency of the enzyme adenosine deaminase (ADA). The deficiency causes an almost complete absence of normal immune function, meaning that children who suffer from it - there are fewer than 20 worldwide - must live in a sterile "bubble" unless the enzyme can be replaced.

Bordignon intends to remove some of the child's T-lymphocytes and insert a normal human ADA gene into them. But the altered T-cells will be put back into the child's body only if current treatment with bovine enzyme fails. Until that time, the altered T-cells will be frozen.

Unlike the United States, European countries have so far viewed somatic gene therapy as just another medical treatment that does not present special moral or ethical problems. There is no equivalent to RAC in Italy or many other European countries. Nevertheless, opposition to genetic engineering may make such experiments difficult in countries such as Germany.

The EC consensus, reiterated at a March 1990 meeting of research ministers at Kronberg in

Germany, breaks down when it comes to germ-line gene therapies. Although the ministers agreed to reject germ-line therapies for the moment, says Peter Lange, head of the section for basic questions in biology and health at the German Research and Technology Ministry, their opinions diverged about whether such therapies will be permitted in their countries in the future. Changing the germ line using genetic engineering will become illegal in Germany at the beginning of 1991.

The EC Commission is setting up a committee to look into the ethical and social implications of human genome analysis (which may also deal with gene therapies) but an EC official in Brussels says that it is up to the member States to regulate the therapies at national level. In the official's opinion, any attempts to ban somatic gene therapy throughout the EC might well fail because of Article 36 of the Treaty of Rome, which allows nations to deviate from Community policy with respect to issues of "public mortality ... public safety and the protection of human life and health". (Source: Nature, Vol. 348, 29 November 1990)

First-ever human gene-therapy surmounts regulatory obstacle course

On Friday, 14 September, shortly after noon, a team led by W. French Anderson, who heads molecular haematology at the National Heart, Lung and Blood Institute (NHLBI), infused a four-year-old girl with about one billion of her own white blood cells into which they had spliced copi of the gene that expresses ade osine deamin e (ADA). The half-hour infusion became the first human gene-therapy experiment to be attempted since 1980.

Anderson's group of clinicians, which includes R. Michael Blaese and Kenneth W. Culver of the National Cancer Institute (NCI), three and a half years after initially seeking permission, finally received approval from:

- NIH's biosafety committee;
- Review boards at both NHLBI and NCI;
- NIH's Recombinant-DNA Advisory Committee (RAC);
- RAC's Human Gene Therapy Committee;
- The Acting Director of NIH, William F. Raub;
- A Food and Drug Administration (FDA) external advisory committee.

The first phase of this experiment, Anderson says, is primarily designed to test for safety. The girl, whose identity has not been revealed publicly, will have her genetic immune deficiency staved off by PEG-ADA - a synthetic form of the missing enzyme that does not degrade immediately when given exogenously, as natural ADA does. This supplementation will continue until it becomes obvious that her gene-transformed T-cells supply enough endogenous ADA to keep her healthy.

Once a month for the first six months, she will get back 0.1 per cent of her transformed T-lymphocytes, to make sure the procedure is safe, and that the cells are actually producing ADA in vivo. Then, the second phase will begin, in which large numbers of T-cells, 10^{30} times the earlier dose will be given. If ADA production is at least 5 per cent of normal, "she will be healthy". Anderson suggests that it will take 18 months to two years to attain this level, although "it may be quicker". After that, he says, "we will submit a new protocol, in which we would withdraw PEG-ADA".

Three other ADA-deficiency children will begin the same protocol within the next two to three months, Anderson says. Within the last month, researchers have identified the genes responsible for three diseases - potential targets for human gene therapy. They are listed in the following table. (Source: Biotechnology Newswatch, 1 October 1990)

Recently discovered disease genes

Disease entity	Gene	Where discovered
Osteoarthritis	Abnormal collagen-2 gene; arginine replaced by cysteine, causing improper coiling of the molecule	Case Western Reserve University, Cleveland; Thomas Jefferson University, Philadelphia; published in the Proceedings of the National Academy of Sciences
Lung cancer	CYP 1A1 gene activated by cigarette smoke to yield aryl hydrocarbon hydroxylase	National Cancer Institute, Bethesda, MD; Johns Hopkins University Medical School, Baltimore; published in the Journal of the National Cancer Institute
Fascioscapulo-humoral muscular dystrophy	FSH gene	University of Leiden, The Netherlands; University of California, San Diego; Collaborative Research Inc., Bedford, Mass.; Duke University, Durham, N.C.; published in The Lancet

"DNA fingerprinting" standards needed

Use of the technique commonly known as "DNA fingerprinting" in criminal investigations has mushroomed: the Office of Technology Assessment (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, defence attorneys have successfully challenged DNA evidence in court by uncovering sloppy procedures and subjective interpretations. Scientists, too - most notably geneticist Eric S. Lander of Harvard University, who provided material for an OTA report - have warned about the lack of rigorous standards.

DNA fingerprinting uses molecular biology techniques developed over the past 20 years to compare samples of DNA taken from a suspect with those found at a crime scene. The DNA samples are cut into fragments by restriction enzymes that cleave the molecules at specific locations. The resulting fragments - theoretically unique to any individual - are separated by gel electrophoresis and visualized through the use of radioactive probes.

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.

Defence attorneys have recently raised the question of whether marriages 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. (Abstracted with permission from Chemical and Engineering News, p. 6, 13 August 1990, by Pamela Zurer. Copyright (1990) American Chemical Society)

Biotransformation Club activities for 1990-1991

Among the activities planned by the Biotransformation Club (BTC) for the period to March 1991 are technical reports on the following subjects: (1) the potential use of catalytic antibodies for biotransformation reactions; (2) the potential impact of protein engineering on biotransformations; (3) biological fluorination; (4) biotransformation for the food industry; (5) immobilization in biotransformation; (6) biotreatment of toxic and hazardous wastes, including nitroaromatics; and (7) anaerobic biotransformations.

In addition, work is in progress on biotransformations by extremophiles and generic problems in organic chemistry. Bimonthly abstracts prepared by the Warwick University Biotransformations Club are available to BTC members. Details from: Sue Armfield, Biotransformation Club, Laboratory of the Government Chemist, Queen's Road, Teddington TW11 0LY or on 081-943 7463. (Source: Biotechnology Bulletin, Vol. 9, No. 8, September 1990)

Pharmaceutical industries changes in the decade ahead

The pharmaceutical industry will be shaped by major forces in the decade ahead. New discoveries, domestic and international competition, economics, service, demographic changes and the public sector are all significant driving factors. A new report by Business Communications Co. emphasizes the global character of the industry. The prime major event will be the forthcoming unification of Europe with its unified science policy, regulations and markets.

The US pharmaceutical market is one of the most diversified in the world, with some 60,000 substances to treat, with varying degrees of success, human diseases. According to the study, US pharmaceutical sales will grow at an average annual rate of 9.6 per cent, increasing from a 1989 value of $US 35,929 million to $US 101,399 million by the year 2000.

As a direct result of the AIDS epidemic in the United States and around the world, AIDS-related drugs will represent the fastest growing segment of the pharmaceutical industry over the next ten years. Experiencing an average annual growth rate of 18 per cent, AIDS drugs will grow from a 1989 level of $US 9,200 million to $US 25,533 million by the year 2000.

On the supply side of the pharmaceuticals market, some of the most important aspects include:

(a) The segmentation of the pharmaceutical industry by therapeutic groups (e.g., cardiovasculars, dermatologicals, etc.) and by the patent status of individual drugs;

(b) The progressive absorption of biotechnologies into traditional pharmacology. This results in increased segmentation, even within individual therapeutic groups;

(c) Global competition existing within segmented markets, exemplified by Bristol Myers/Squibb, which is the leader in the cardiovascular category in many European countries and in the cancer category in the USA.

The report "New Horizons in the Pharmaceutical Industry: A Global Perspective" is distributed in Europe by RauCon GmbH, P.O. Box 1069, D-6912 Dielheim, Germany (Phone: +49 (6222) [illegible]562, Fax: +49 (6222) 74884) and costs $US 2,450. (Source: RauCon News Release, 16 November 1990)

Biohazard testing becomes an increasingly important industry in the 1990s

Biohazard testing, in the forms of human toxicity testing and environmental effects testing,

has a pivotal role in industry and commerce, as the results often contribute to the initiation of standards, regulations, remediations, controls and material substitutions. Labelling a chemical "toxic" has major implications and biohazard test systems are therefore becoming increasingly complex and subtle as new types of toxicity are recognized.

The biohazards industry is likely to undergo a period of profound change in the 1990s, according to Biohazard Testing: The Business, The Issues, The Applications (C-126), a new report by Business Communications Co. (BCC).

At the same time, biohazard testing will become important to a wider range of industries and product categories. Overall, the volume of biohazard testing in the US is expected to grow at an average 2.0 per cent annual rate, increasing from about $679.6 million in 1990 to approximately $750 million by 1995. This growth will be fuelled by the testing of new chemicals, with some contributions from re-visits to old chemicals previously tested and old chemicals being tested for the first time.

BCC predicts that sales of products for biohazard testing in the US will increase by about 1.2 per cent a year, from a total of $133.7 million in 1990 to about $142.1 million by 1995. Conventional animal testing, as measured in terms of animal supply, is expected to increase at a modest 1.1 per cent, from about $130.9 million of mammalian animal sales in 1990 to approximately $138.2 million by 1995.

US sales of in vitro toxicity testing products will increase at an average annual rate of 3.8 per cent from $2.2 million in 1990 to about $3.2 million by 1995. During this period, sales of conventional products are expected to decline by an average 0.6 per cent a year, from about $1.7 million in 1990 to $1.65 million in 1995, resulting from an anticipated contraction in the customer base for conventional mammalian cell lines.

BCC research suggests that newly emerging in vitro testing products will grow at an extraordinary 24.8 per cent growth rate, from a 1990 level of $0.5 million to $1.5 million by 1995. The animal rights issue, combining with the application of new technologies, is contributing to this high expected growth. At the same time, the validation process that the new in vitro products must undergo to find final acceptance is slowing the pace at which they can penetrate target markets.

Envirotoxicity testing, addressing the effects of chemicals on fauna and flora, is only a minor contributor in 1990, providing about $0.6 million in sales of non-mammalian animal species and plants. BCC projects that envirotoxicity testing will grow at only about 2.8 per cent in the next five years, reaching about $0.7 million by 1995, despite concerns over chemicals in the environment. Details of the report, priced at $2,850, from: Business Communications Co., Inc., 25 Van Zant Street, Norwalk, CT 06855, USA or on +1 (203) 853-0348. (Source: Biotechnology Bulletin, Vol. 9, No. 10, November 1990)

International course for food fermentation technology organized by the Research Institute for Food Resources, Korea University, Seoul, Korea (1 August 1991 to 31 July 1992)

This international course is a part of the activity of the UNIDO project on "Industrialization of lactic acid fermentation technology of cereals and its dissemination to developing countries". The purpose of the training programme is to introduce the traditional fermentation technologies of Korea to the scientists of the other regions of the world and to illustrate the way to industrialize tradtional processes by using the modern concept of biotechnology.

The course includes lectures, laboratory works and pilot-plant practices. The lectures are of post-graduate level given mainly by the faculty members of the College of Agriculture, Korea University. Each trainee will be engaged in different laboratories of the College of Agriculture for his/her collaborative researches.

The pilot-plant practices will be carried out at the Korea Food Research Institute. The language of the training is English.

Further information regarding fellowships, etc. may be had from:

Professor Cherl-Ho Lee
Department of Food Technology
Korea University
1 Anamdong, Sungbukku
Seoul, Korea, 136-701
(Fax: 82-2-9275201, 82-2-9225820)

Summary of UNEP/MSDN Workshop on an Information Resource for the Release of Organisms into the Environment

The aim of the Workshop, which was supported by the Environmental Protection Agency (UNEP), UNIDO and the US Department of Agriculture in the USA, Environment Canada and the Biotechnology Directorate of the European Commission, was to consider the needs and specifications for an information resource on the release of organisms into the environment. Because of travel restrictions the Workshop took place simultaneously in Vienna and Washington between 10-15 March 1991. It brought together experts in microbiology, release regulatory matters, database and network development and management. The meetings were linked by electronic mail, pre-recorded video tapes and teleconferences, as well as by an on-going computer conference to which those unable to participate at either site could contribute.

There was considerable agreement between the two groups on the main topics that were discussed and the following recommendations were made:

There was a need for an integrated information resource on the introduction of organisms into the environment;

The resource should be international in scope and cover both non-modified and genetically modified organisms;

The resource should be a distributed network linking both existing resources and new databases that are developed to fill gaps in information; the separate data elements should be linked by gateways where appropriate and usage should be facilitated by the development of interfaces;

The resource should be flexible, to accommodate different data systems already in operation (that may have well established distribution policies) and adjust to new circumstances that may arise;

The resource should be not-for-profit and data should be made freely available without restriction as far as possible;

The resource should be reliable and internationally accessible;

Regional help desks and training should be important parts of the resource.

The need for an inventory of existing relevant information resources was unanimously agreed and the MSDN will take steps to initiate this using the expertise of the CWG for the purpose.

The MSDN should initiate arrangements for the setting up of a Steering Committee (SC) on behalf of the Workshop. The Workshop emphasized that the SC should include the users of the resource and ensure geographical representation. The SC should establish any working groups needed to decide the key issues in realizing the recommendations of the Workshop.

The Core Working Groups (CWG) at each site have agreed that MSDN should continue its present administrative role by:

Preparing a summary document for wide distribution;

Preparing the Proceedings of the Workshop for UNEP publication;

Initiating the establishment of a Steering Committee;

Initiating the preparation of an Inventory of existing resources;

Arranging a follow-up meeting (possibly at REGEM2 in Nottingham, UK, in August 1991);

Finalizing recommendations to UNEP for the establishment of an IRRO.

The groups both felt strongly that the success of the resource depended on the close collaboration between the scientific and regulatory communities. Moreover, they agreed that the initiative was very relevant to the biodiversity programmes presently under discussion and the recommendations of the Workshop should be conveyed to the UNCED Conference in 1992 and to other related programmes under consideration, such as those of IUBS and the CEC.

B. COUNTRY NEWS

Australia

Australian medical discovery

An Australian discovery, which could lead to the successful treatment of a serious disorder in the blood of cancer patients undergoing radio- and chemotherapy, has resulted in a significant commercial arrangement between Sandoz Pharma Ltd. of Switzerland and AMRAD Corporation Ltd. of Melbourne. The research collaboration and licence agreement, initiated by AMRAD, guarantees that at least $2 million worth of R&D will be conducted in Australia over the next two years.

The collaboration is based on a world first, the discovery of the LIF (Leukaemia Inhibitory Factor) by scientists at The Walter & Eliza Hall Institute of Medical Research (WEHI), renowned for its cancer research. Named to describe its first identified function as a factor modifying leukaemia cells, LIF is thought to have the potential for a number of major human therapeutic applications. Foremost amongst its effects is the potent stimulation of the generation of platelets. The joint research between Sandoz Pharma and AMRAD will concentrate initially on LIF's effect on platelets in blood. A platelet deficiency, thrombocytopenia, is commonly caused by cancer treatments such as radio- and chemotherapy and the research may result in a treatment for the condition. (Source: Australian Journal of Biotechnology, Vol. 4, No. 4, October 1990)

China

Law to boost R&D

China's State Science and Technology Commission is drafting a law to boost the research and development of science and technology. The new law will set out a framework detailing planning, funding and R&D structures. The Commission has taken into account that even if companies make profits from projects, these are often not very large, and it will not be penalizing profit-making companies by taking away State funding. (Source: European Chemical News, 10 December 1990)

Ethiopia

Farmers conserve seeds in Ethiopia

Since 1988 the Plant Genetic Resources Centre, Ethiopia (PGRC/E) has been conducting a farm-based plant genetic resources programme in close co-operation with peasant farmers, agricultural extension agents, scientists and organizations working in agricultural development in Ethiopia. The programme includes on-farm landrace conservation, maintenance and utilization of landrace elite populations in stress prone areas of Ethiopia. It is a crucial part of the "Seeds of Survival" Programme.

The on-farm landrace programme was designed primarily to help the Ethiopian farmers retain their crop diversity. Major cultivars threatened by extinction are protected and their performance improved allowing the farmers to maintain a sustainable source of planting material and use the seed of their choice. The long established skill of stabilizing crop production by nurturing a broadly adapted genetic base is being protected and further developed along with the seed.

As a long-term protection measure of the country's rich crop genetic heritage the programme is added, as an integral part, to the overall genetic resources conservation strategy. It provides a reassuring back-up for the gene bank (PGRC/E). A similar programme focusing on wild plants with potential food value, traditionally known as "famine crops", is envisaged for areas affected by extreme droughts and other stresses. (Source: African Diversity, No. 4, December 1990)

European Community

BST ban could be extended by European Commission

As Europe's milk lake deepens, the biotechnology industry is increasingly concerned that one of its flag-ship products - the genetically engineered dairy hormone bovine somatotropin (BST) - will be stalled again by the European Commission. A

key decision is also awaited from the European Committee for Veterinary and Medical products (CVMP), which was instructed last year not to decide until November 1991.

In the mean time, the EC agricultural commissioner announced that he would seek an extension of the "temporary" ban on the use of BST, which was imposed last year to allow time to study the health effects of BST.

Scheduled to expire this December, the ban could be extended to next spring, this time to assess the likely impact of BST on European milk markets - already hit by substantial overproduction. Under pressure from Europe's small farmers, the Commission has increasingly been forced to consider BST's socio-economic impacts.

Bovine somatotropin (BST), the milk yield-boosting hormone, received a major blow last month when the UK Veterinary Products Committee (VPC) refused to license Monsanto's BST product for veterinary use here.

It was understood that the VPC also provisionally refused a licence for a second BST product, developed by the other major US manufacturer, Eli Lilly.

The VPC said that while it was satisfied about the human safety aspects of BST's use, it remained concerned about "some pharmaceutical aspects of the product (and) with aspects of the safety of treated animals".

Monsanto, whose trials of BST in the UK are now in their fifth year, said that it would appeal the decision - and noted that it was confident that it could satisfy the VPC. (Source: Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Political hostility is hitting EC biotechnology R&D

The European Community is falling behind in biotechnology research and development mainly due to political hostility, according to a new report written by public policy consultancy, Prima Europe, commissioned by Eli Lilly International.

The report, The Case for Biotechnology, coincides with a Gallup survey of public attitudes towards biotechnology, which canvassed 3,166 people in the UK, France, Germany and Italy.

The survey found that, on average, only 4 per cent of the population in the four countries would like to see a total ban on biotechnology research, while 49 per cent want to see a partnership between government and industry in setting standards for biotechnology and only 37.5 per cent of people want strict regulatory control.

The danger of eugenics and environmental harm are the two greatest causes of concern.

The report, which has reportedly been used to lobby members of the European Commission, states that at the moment, Europe's own biotechnology entrepreneurs and corporations prefer to invest in the US: biotechnology is developing faster in the US and Japan than in Europe. It says the reason is a greater political hostility to biotechnology in the Community, reflected in a regulatory system which the biotechnology companies regard as incoherent and hostile.

The world market for biotechnology-derived products and processes is predicted to be worth more than Ecu 80 billion ($108.8 billion) by the year 2000. The report says: "For its own future prosperity, Europe will need to participate fully in this world-wide market by contributing its own research and development".

It calls for a reasoned case-by-case licensing of each planned release of genetically engineered product by an advisory committee that includes public representatives, as well as industry and government.

The process should be along the lines of the US system of assigning low, medium or high rating to the product, depending on the nature and experience of the product. (Source: European Chemical News, 10 December 1990)

France

Foetal cell transplants

A French national ethics committee for life sciences and health has overturned its ban on the use of foetal brain tissue grafts for medical treatments.

This means that a team of doctors at the Henri-Mondor hospital in Créteil, south of Paris, will now be able to carry out pilot operations to treat patients suffering from Parkinson's disease.

This kind of treatment is now authorized in some other European countries including Britain, Spain and Sweden, but is allowed in the United States, where there is a powerful anti-abortion lobby, only so long as federal funds are not used.

In October 1989, the ethics committee ruled that more research was needed before foetal tissue could be used for this kind of operation. At that time, foetal dopamine-producing cells were used to enhance the effects of adrenal tissue extracted from the patient and reinjected into the area of the brain where dopamine is produced.

This is a very dangerous procedure. But new research in Sweden has made the operation safer. (Source: Nature, Vol. 348, 20-27 December 1990)

Genome a la carte

The French Government is to strengthen the country's research into mapping the human genome, which at present involves 500 research staff at almost 70 laboratories in France. Herbert Curien, the French research minister, announced plans to set up a national genome research programme. He also vowed to double state spending on genome research to £10 million a year by 1992.

He said that the Government plans before the end of this year to set up a national committee for co-ordinating the programme, drawing up investment programmes for each of the participating centres, and representing France in deliberations about pan-European and international genome projects. The committee will also encourage the marketing of new

products emerging from genome research, and is to consult regularly with France's ethics council. (Source: New Scientist, 27 October 1990)

Biotechnology sets the pace in Europe

Just eight years after the Government launched its Mobilization Programme for Biotechnology under the auspices of the Ministry of Research and Technology, France has become a formidable international force in the fiercely competitive biotechnology marketplace.

There are a number of reasons why France was not present at the beginning of the revolution, but among the most important were bureaucratic inertia and a lack of communication between industry and academia.

The Mobilization Programme created an executive council made up of scientists and representatives from government and industry. The first priority was to promote genetic engineering and to be able to produce human proteins.

In addition to giving centralized direction to French efforts in biotechnology, the Mobilization Programme provided a major infusion of cash. At its height, direct government support reached almost 2 billion francs per year, administered through national research agencies such as the Institut National de la Santé et de la Recherche Médicale (INSERM) and the Centre National de Recherche Scientifique (CNRS).

Although the Mobilization Programme provided a tremendous stimulus, the biotechnology industry in France - and to a large extent elsewhere in Western Europe - has developed along somewhat different lines than it did in the United States.

French biotechnology is largely dominated by the giant multinational firms Rhône-Poulenc, Sanofi and Roussel Uclaf, either directly or through subsidiaries. There are nevertheless some significant exceptions although one of the most successful of these - Transgène, an independent company based in Courbevoie and Strasbourg - is currently discussing a merger with Institut Mérieux of Lyon, which is in turn controlled by Rhône-Poulenc.

Transgène also provides an example of the kind of collaboration between research scientists and industry that was rare 10 years ago. Together with Institut Mérieux, Transgène is working with André Capron of the Pasteur Institute of Lille to develop a vaccine for schistosomiasis, a serious disease that affects about 200 million people, largely in tropical regions of Asia, South America and Africa. Over the past several years Mr. Capron's research team has identified a number of proteins on the worm's surface that stimulate the production of antibodies.

Transgène has used genetic engineering techniques to clone the genes responsible and produce the proteins, which may soon form the basis for clinical trials of a human vaccine.

Such collaborations had also led to commercial agreements between academic research institutions and industry. Most notable was the formation last year of Pasteur-Mérieux Serums and Vaccines by the Institut Mérieux and the Pasteur Institute in Paris.

Pasteur-Mérieux has already introduced a genetically engineered vaccine for hepatitis B, and is working on a number of other projects, including an AIDS vaccine. (Source: International Herald-Tribune, 31 October 1990)

Germany

Germany passes Gene Law

On 24 October, the Bundestag passed what was billed as the world's first law aimed at controlling genetic engineering of human embryos - and at preventing the mixing of human genes and animal genes (or any other genetically different tissues) for research purposes.

Artificial fertilization of eggs is permitted, but only enough egg cells may be taken at any one time to carry out a single treatment, except in exceptional circumstances.

The law also rules out any method of predetermining the sex of a child, except in cases of serious hereditary illnesses. (Source: Biotechnology Bulletin, Vol. 9, No. 10, November 1990)

Genetic projects given go ahead

Hoechst and its subsidiary Behringwerke have received permission to proceed with controversial gene-splicing projects. Hoechst will continue work on its three-stage complex to produce recombinant human insulin, and Behringwerke will build its facility to produce erythropoietin from manipulated mouse cells.

The insulin project, planned since 1984, suffered setbacks after pressure from environmentalists.

A court in Frankfurt has now overturned an interim ruling made in November 1989 and reinstated Hoechst's permit to operate stage one of the project and build stage two. It hopes to begin production early in 1991. Behringwerke, which stopped work in November of last year, hopes to begin production in autumn 1991. Both companies already face competition for gene-splicing drugs. (Source: Manufacturing Chemist, November 1990)

New genetic engineering programme adopted

The University of Bochum has tested a particularly interesting example of what biotechnological methods can achieve: Whereas some 10,000 mice were previously required to produce one kilo of monoclonal antibodies to protect the human body from antigens, scientists have succeeded in using a biotechnological process to produce monoclonal antibodies, or in other words homogeneous proteins that protect the body-invading antigens, in a glass container. Launching the federal Government's "Biotechnology 2000" research and development programme, Dr. Heinz Riesenhuber held up this device, known as the "glass mouse", as a prize exhibit. Under this programme, the federal Government will subsidize research and development on biotechnological processes and products that involve genetic engineering over the period from 1990 through 1994. In this four-year period, the BMFT (Federal Ministry of Research and Technology) will spend DM 1.5 billion. This is half as much again as in the last five years.

According to the Federal Research Ministry, there are to date more than 1,000 laboratories, working with genetic engineering methods in the FRG and more than 10,000 worldwide. At present, biotechnology's greatest significance lies in pharmaceutical research. In the FRG 22 companies, 85 per cent of which are classed as small and medium-sized businesses, have introduced bio-technical methods in the plant breeding, food technology, and chemical product and engineering sectors. The federal research minister expects further important innovations in agricultural and environmental technology applications within the next few years. (Source: Wissenschaft, Wirtschaft, Politik, 5 September 1990)

Italy

Biotechnology laboratory network planned

One of the projects promoted by CIB, the Inter-university Biotechnology Consortium that includes 15 Italian universities, is a laboratory network to carry out biotechnology research oriented towards industrial applications.

The first laboratory will be inaugurated in Trieste by the end of the year at the Trieste Research Area, where the consortium has its administrative and operational headquarters. It will also act as an interface between Italian universities and UNIDO's Trieste-based International Genetic Engineering and Biotechnology Centre, directed by Arturo Falaschi.

The goal of the consortium's scientific committee is for each laboratory to take a multidisciplinary approach to specific issues, with particular attention to applications. Initially, the Trieste laboratory will be involved in gene mapping and the development of automated systems for DNA sequencing, so that these will rapidly become marketable products of industrial interest.

The consortium intends to act as a co-ordinating centre whose goal is to promote the development of research groups until they reach a sufficient size. Unlike the traditional branches of study in Italian universities, it has a "transversal" structure that consists of research groups of biochemists, genetic scientists, chemists, engineers, pharmaceutical chemists, biologists, etc. It will act as an interface with the industrial world. The initiative is based along the same lines as those of various other European countries.

The first part of the funding, allocated within the 1988 budget soon after CIB's establishment, and by the centres involved in the consortium, was used primarily to equip all the centres with the new, large machinery required to carry out research.

Other initiatives under way include setting up a data base to collect information on the size, projects, and scientific productivity of over 150 operational units. If a company wishes to start a research programme, it can use the data base and the consortium to obtain the necessary contacts. (Source: Italia Oggi, 26 June 1990)

Aphrodisical food for exuberant silkworms

With a secret but natural feed mixture, silkworms will be able to reproduce 25 times a year instead of the traditional four.

The project is being developed by the Ratti Group and ENEA (Italian Committee for R&D of Nuclear and Alternative Energies), and is financed equally by both. By next year, a prototype plant to artificially breed the very precious little animal on an industrial scale will be constructed at Guanzate (near Como, where the Ratti plants are located). The biofactory will produce eight metric tons of cocoons annually, the equivalent of 1.2 metric tons of raw silk. Italy currently imports 4,400 metric tons of raw silk per year, 3,100 of which comes from China.

Alternative solutions had been studied and tested for some time, until September last year, when Ratti and ENEA announced the launch of the production phase of the "silkworm breeding project". The entire Como textile industry places great hope in its success for several reasons: the crisis that has affected certain areas of the Italian textile industry; the Chinese policy of export quotas for raw silk (to "boost" its own textile industry), and the recent and heavy increase in prices at origin.

The problem was that the silkworms are fussy, always accustomed to eating mulberry leaves, and possibly only those without dressing. A dangerous dietary dependency - as was demonstrated by last year's fall in production due to the careless use of a certain insecticide in orchards.

A Japanese solution was already on the market. Except that this feed, which was recently patented in Japan and is the only alternative feed in existence, has the double disadvantage of being expensive and usable only during the "weaning" period. With the Japanese feed, the use of mulberry leaves in the diet is reduced but not eliminated.

Strengthened by the expertise acquired in breeding insects for use in agriculture, ENEA developed a particularly interesting solution that combines three levels of innovation: a semi-synthetic feed which has sugar and soya as its basic components; the use of silkworms that have been adapted to the artificial diet, and the use of automated plant technologies. Although the feed costs one and a half times more than mulberry tree leaves, it permits a fivefold increase in reproduction.

The Ratti Group will be responsible for this first operational phase of the project, lasting from three to five years, and will be involved in equipping and then managing the plant at Guanzate. A research laboratory will work together with the biofactory to monitor the quality of production, while a technological hall will prepare the feed and update the technical methods used.

One challenge remains before complete independence from overseas is achieved. It has not yet been possible to obtain the egg of a national polyhybrid silkworm, although experiments continue to adapt several polyhybrids developed by the Ministry of Agriculture's experimental silkworm rearing centre to the artificial feed. In the mean time, an agreement has been reached with the Japanese for the supply of a polyhybrid egg that can produce cocoons of a consistent quality and quantity. (Source: Italia Oggi, 3 July 1990)

Japan

Joint marine biotechnology projects

The Marine Biotechnology Institute will launch joint research projects with the Woods Hole

Oceanographic Institute (WOI), the Australian Institute of Marine Science (AIMS), and the USSI Heliosynthesis Laboratory (France). The research areas include thermal-resistant bacteria found in the waters around hydrothermal vents (with WOI), CO_2 fixation by calcerous algae and the mechanism of symbiosis between the algae and corals in the Great Barrier Reef (with AIMS), and microalgae with high potential CO_2 fixation (with USSI).

The Marine Biotechnology Institute is an R&D company specializing in marine organisms; it was established with the support of 24 companies as well as the Agency of Industrial Science and Technology. The two facilities in Kamaishi (Miyagi Prefecture) and Shimizu (Sizuoka Prefecture) started research in July, and a research vessel is also operating. The Institute is expected to play an important role in facilitating international research co-operation. (Source: Bio/Technology, Vol. 8, October 1990)

Research project on genome structure of crops

Trying to catch up to US and European research efforts in this field, the Ministry of Agriculture, Forestry and Fisheries will launch a long-term research project on the genome structure of crops and commercially important animals. The project aims to establish the proper scientific background for applying biotechnology to food production, such as the development of improved strains with favourable phenotypes including disease resistance and high nutrient content. The project is divided into three periods: the first, which will start in the next fiscal year, will focus on sequencing the entire genome of rice; the second, on crops and trees; and the third, on livestock and important aquatic organisms. Each period is expected to be 7 to 10 years; with a total budget of 20 to 30 billion yen; this is the Ministry's largest research project to date. National research institutes are involved in the project, together with industrial and academic sectors. The establishment of a new research centre is also being considered. (Source: Bio/Technology, Vol. 8, October 1990)

Kenya

Seed conservation at community level

Introduction of foreign plant species, cash crop economy and socio-economic pressures have led Kenyans to neglect their own plant genetic resources. Realizing the threat to Kenya's genetic diversity the Kenya Energy and Environment Organisation (KENGO) has taken up the active protection of indigenous plants.

KENGO's first initiatives, starting in 1982, were an awareness-raising campaign and the collection of ethnobotanical data on some 120 economically important indigenous trees. A wealth of information on traditional uses came from elderly people. The data were compiled in the Resource Book of Indigenous Trees in Arid and Semi-arid Areas of Kenya.

The Seeds and Genetic Resources Project, which included the promotion of simple propagation techniques for indigenous seeds and training in seed collection and handling, followed. Community oriented booklets assisted the work: A Pocket Qirector of Trees and Seeds in Kenya; Seeds and Genetic Resources in Kenya; National Expedition on Genetic Resources and Habitats; and How to Collect, Handle and Store Seeds.

A research project on ex situ conservation of economically important indigenous trees was carried out jointly with the Jomo Kenyatta University College of Agriculture and Technology. It included the development of field gene banks and propagation techniques for 35 species. In collaboration with Kenyan research institutions indigenous fruit and vegetable samples from various parts of Kenya were assessed for their nutritional value.

For the near future a Plant Biodiversity Conservation Programme involving the establishment of plant sanctuaries, community seed banks and information campaigns on the need for in situ conservation of threatened natural habitats is being planned.

Some facts from KENGO's vast programme experience are of particular interest, e.g. the high nutritional quality of some local fruits and vegetables or the fast growth of some indigenous trees. Erosion of culture and traditional values had led to traditional food plants being associated with poverty. This and the farmers' need for economic returns for conservation activities, which can compete with the returns from introduced crops and cash crops, are among the major obstacles KENGO has to deal with. (Further information from: KENGO, P.O. Box 48197, Nairobi, Kenya) (Source: African Diversity, No. 4, December 1990)

Netherlands

Concern over mammalian cell contamination

The Dutch Medicines Control Authority has not approved Ares-Serono's (Geneva, Switzerland) mammalian cell-derived human growth hormone (hGH), which is approved in all other European Community member States, other parts of Europe, and South America.

The rationale for the decision is that other hGH products, not produced in mammalian cells, are already available. The Dutch concern is over possible risks from viral or other agents in the cell lines or medium components, in particular bovine spongiform encephalopathy (BSE).

The scientific basis of the issue will be discussed at a forthcoming meeting of the Committee for Proprietary Medicinal Products (CPMP, Brussels). While the initial focus was on the possible presence of the BSE agent in the serum used for mammalian cell culture, the discussion will likely encompass other animal-derived media components, such as peptones, which are also used in bacterial and fungal media. (Source: Bio/Technology, Vol. 8, October 1990)

Saudi Arabia

An embryonic cure

In Saudi Arabia, hundreds of years of desert isolation have led to a tradition of marrying into your own family. This has inevitable consequences, one of which is the prevalence of inherited diseases that would otherwise be rare. So numerous are such genetic disorders that the Saudis have opened an Inborn Errors of Metabolism Centre at King Faisal Hospital in Riyadh, where Dr. Pinar Ozand, a Turkish-American paediatrician, leads the research.

One of the nastiest types of condition facing Dr. Ozand and his colleagues goes under the name of lysosomal storage disease. It establishes itself

early during a foetus's development. An affected infant cannot break down wastes properly because it does not produce enough of the right sort of enzymes - and as a result will probably die between the ages of two and four. Although such conditions can be diagnosed long before birth, Islamic law and tradition does not allow affected foetuses to be aborted.

In Saudi Arabia the frequency of such conditions is 5-10 times higher than the world average of one in 100,000. The most prevalent in the Kingdom is Sandhoff's disease, affecting one in every 2,000 people. It is closely related to Tay-Sachs. In Sandhoff's, an affected child lacks one of two components of an enzyme called hexosaminidase. As a result the child cannot digest certain sugar-containing compounds, which travel instead to the brain; by the age of one it is blind and demented. Children with Tay-Sachs disease lack the other component of the enzyme.

There have been attempts to treat storage disease after birth with bone-marrow transplants; there are no proven therapies. But recently two doctors in America performed the first transplant operation in the womb to overcome storage disease before birth. The baby has just been born. Dr. Esmail Zanjani of the University of Nevada in Reno and Dr. William Krivit of the University of Minnesota in Minneapolis, who has done post-natal work on storage disease for many years, are evaluating the results and think that further use of their procedure is just a few months away. Their technique is to take certain "stem" cells from the livers of aborted foetuses and inject them into the belly or blood of a foetus with storage disease at about the 12th week of its development During gestation these cells spread from the liver to bone marrow and thence to all organs, including the brain. A foetus with healthy transplanted stem cells will produce enough of the crucial enzymes.

As well as Sandhoff's disease several other storage diseases should be treatable in the same way; the only differences between the diseases are in the particular enzymes affected. The method is an improvement over post-natal bone-marrow transplants because it should be able to stop the disease before it has set it, and should cost considerably less. An ethical committee at King Faisal Hospital has still to approve the procedure. If it is approved, frozen cells could perhaps be flown in from Europe or America. Doctors at the hospital feel that the desperate nature of the disease would justify such measures. (Source: The Economist, 17 November 1990)

United Kingdom

Key project renews hope for Brazil's poorest

Three botanists from the Royal Botanic Gardens at Kew outlined ambitious plans to reverse the ecological crisis in north-east Brazil. At the first Anglo-Brazilian Environment conference in Brasilia, Raymond Harley, Simon Mayo and Charles Stirton unveiled Projeto Nordeste, "Plants for People".

The project, which needs to raise £4.5 million, aims to improve the way of life of local people and so prevent massive migration to the overcrowded cities of the south and into the Amazon. The 10-year programme will involve scientists from Kew and five Brazilian institutions.

Thirty per cent of the country's population lives in the nine north-eastern states, which suffer from devastating droughts. Many of the miners and colonists that have settled in the Amazon Basin come from the north-east.

Yet the region is biologically the richest part of Brazil, with between 15,000 to 20,000 species of plants. Four hundred and fifty years of colonization have devastated all the ecosystems of the north-east so that they no longer support even the basic needs of the local people, such as permanent supplies of water. Half the region is covered by semi-arid forest, the Caatinga. Virtually all the species in the Caatinga have some useful properties, and the programme concentrates on identifying these qualities. Overall, it aims to slow down the spread of desert by repairing what is left of the indigenous forest and improving the productivity of the land.

The botanists will identify and domesticate species of trees and shrubs that are adapted to dry conditions, and which have plenty of uses locally - as food and animal fodder, fuel, timber or medicine. The information they collect will be gathered together in a data base of indigenous plants, that will be available to researchers throughout the world. (Source: New Scientist, 3 November 1990)

Data base for plant species

Botanists are compiling a computer data base of plant species names despite the chronic shortage of funds for taxonomic research. A meeting held on 12-13 November at the Royal Botanic Gardens, Kew, attracted delegates from 45 institutions to discuss Kew's own data base initiative, the Species Plantarum Project (SPP). Grenville Lucas, keeper of the Herbarium at Kew, thinks that the SPP will cost some $10-20 million, but the need for a taxonomic data base is so great that botanists simply cannot wait for funds.

The first five-year phase of SPP will be a simple checklist of all 250,000 species of seed-plant, ferns and mosses. The second phase will be an in-depth treatment of basic plant taxonomy and biogeography, a taxonomic equivalent of the Human Genome Project. When complete, the SPP should supplant its eighteenth-century forebear, Linnaeus's Species Plantarum. SPP is just one of several ideas designed to cater for an increasing need for up-to-date taxonomic information. (Source: Nature, Vol. 348, 22 November 1990)

Catalytic cash input

Research on catalytic antibodies in the UK has been given a significant boost, with the announcement of a new project at York and Strathclyde universities. Funded under the LINK biotransformations programme, half of the money comes from seven companies: British Gas, Sandoz, Shell/Biocode, ICI, Rhône-Poulenc and Eli Lilly.

The announcement comes at a time of heightened interest in catalytic antibodies, or "abzymes", which were first produced four years ago by researchers in the USA.

Research on abzymes is now being conducted in at least five universities in the UK: York, Strathclyde, Sheffield, Cambridge and Queen Mary College, London. The Sheffield group, under

Michael Blackburn and Denis Bunton, have already received £0.04 million under the LINK programme, with Roche and Celltech providing the industrial half of this. Meanwhile, Glaxo, a conspicuous absentee from the list of companies involved, is believed to be considering its own tie-in with the Sheffield group. (Source: Chemistry and Industry, 15 October 1990)

USA

Biotech promising

Sales of biotechnology products in the US will almost reach the $2 billion mark in 1991, according to Consulting Resources of Lexington, Massachusetts and will rise to $5 billion by 1996 and $14 billion by 2001. This would represent an average annual growth rate of 22 per cent.

The human therapeutics area is projected to remain the largest in the sector, with sales rising from $1.2 billion next year to $7.7 billion in 2001.

However, contaminant monitoring is projected to grow by 45 per cent per year and agriculture by 42 per cent per year, taking sales to $200 million and $2 billion, respectively, in the period. (Source: European Chemical News, 5 November 1990)

Biotech drug approved

A Food and Drug Administration advisory panel recommended approval of Immunex Corporation's genetically engineered drug for use in bone marrow transplantation and as a treatment for some forms of cancer and other diseases. The recommendation of the biological response modifiers advisory committee is a major step towards FDA marketing approval for GM-CSF (granulocyte macrophage colony stimulating factor).

The drug will be available for distribution when the agency completes its own review of the product licence application, and clears the product for sale in the US under the trademark "Leukine".

GM-CSF is a genetically engineered version of a human protein that promotes growth and differentiation of white blood cells called granulocytes and macrophages. In addition to bone marrow transplantation, the drug is being investigated for reversing neutrophenia, a white blood cell deficiency in cancer patients treated with chemotherapy. (Source: Chemical Marketing Reporter, 17 December 1990)

AIDS research

Those still optimistic that the war against AIDS will soon be over need only look to the US National Institutes of Health (NIH) for a reminder of the long haul ahead. On the assumption that no end is in sight, NIH announced that they were transferring more than $2 million of their AIDS research funds into training grants to encourage about 60 new scientists to join the field. The funding, which will be distributed to post-doctoral fellows in 14 US universities and research institutes, is the first such effort to ensure that there will be a continuing supply of researchers in the years to come. (Source: Nature, Vol. 347, 25 October 1990)

BST gets clean bill of health

Milk and meat derived from cows treated with a synthetic version of bovine somatotropin, otherwise known as growth hormone, are safe for human consumption.

But despite the approval from experts at a National Institutes of Health (NIH) technology assessment conference, the more than five years of controversy surrounding bovine somatotropin is unlikely to end here. The drug has yet to be approved for commercial use by the US Food and Drug Administration (FDA) and the outcome of several investigations into scientific misconduct involving FDA and the drug companies are still pending.

Despite the reluctance of NIH to become involved in regulatory matters, NIH convened the ad hoc 13-member panel of medical and veterinary experts at the behest of Congress.

The NIH panel concluded that the overall nutritional composition and quality of milk and meat from rbST-treated cows is equal to that of untreated cows and that the health of dairy cows is not appreciably affected. (Extracted from Nature, Vol. 348, 13 December 1990)

Calgene asks FDA opinion on food gene

Calgene Inc. has requested that the Food and Drug Administration issue an advisory opinion regarding the use of a gene in the production of genetically engineered plants.

Calgene says it is the first submission to FDA requesting an evaluation of a component of genetically engineered plants to be consumed directly as whole food. The submission asks for an assessment of the kan(r) gene when used to produce genetically engineered tomato, cotton and rapeseed plants.

The company believes FDA approval of the gene would simplify the future approval of genetically engineered food crops. Calgene plans to file future FDA petitions for such crops and expects the agency will review these alongside the gene petition.

Selectable markers are commonplace in the biotechnology industry and have been used in generic research laboratories for many years. In particular, kan(r) is used as a marker in human clinical trials with genetically engineered human cells. (Source: Chemical Marketing Reporter, 3 December 1990)

C. RESEARCH

Research on human genes

Agent alkylates specific DNA segment

Chemists at the California Institute of Technology have synthesized an oligodeoxy-ribonucleotide equipped with an electrophile at the 5'-end that binds to double-helical DNA by triple-helix formation and alkylates predominantly at a single guanine base adjacent to the target DNA sequence in high yield. Chemistry professor Peter B. Dervan, who has pioneered the use of oligonucleotides for sequence-specific recognition of double-helical DNA, and graduate student Thomas J. Povsic attach a bromoacetyl group to an oligonucleotide designed to bind through triple-helix formation to a 19-base pair sequence in DNA. They reasoned that the reaction of the electrophilic carbon of the bromoacetyl group with the N-7 of guanine would result in covalent attachment of the oligonucleotide to the duplex DNA. Treatment with base would cleave the

DNA backbone at the alkylated guanine. To test the specificity and yield of the reaction, three consecutive guanine-cytosine base pairs were incorporated at the 5'-side of the target sequence. The chemists observed cleavage at the second of these guanines at greater than 87 per cent yield. The results suggest that modification of a single base within chromosomes using strictly chemical methods should now be possible. (Reprinted with permission from Chemical and Engineering News, p. 17, 10 December 1990. Copyright (1990) by the American Chemical Society)

Gene assists spread of breast tumours

French researchers have identified a gene which offers important clues to understanding the way that breast tumours are able to spread. The discovery could lead to therapies to halt the invasion of healthy tissue by the tumour cells.

Breast cancer kills around 15,000 women a year in Britain. It is not the primary tumour itself that proves fatal, but the spread of malignant cells to other organs. If scientists could pinpoint the substance that enables breast tumours to invade tissue, they could devise ways to block its action. But few teams have looked beyond the malignant cells themselves.

Now Pierre Chambon and his colleagues at the Louis Pasteur University in Strasbourg have studied the so-called stromal cells that make up a network of connective tissue around the tumour cells. They took samples of stromal cells from 30 invasive tumours and compared them with those from benign tumours, using DNA probes.

One gene was active in all the cancerous samples but none of the benign ones. And in the cancerous samples, the gene was active only in the stromal cells close to the invading part of the tumour. The gene may have to be expressed for breast cancer to progress, says the team.

The action of the gene produces an enzyme, which the team named stromelysin-3. It is a new member of a family of enzymes known as the metalloproteinases. Scientists already know that these enzymes enable certain other types of tumour to spread.

What makes the gene active? The researchers believe that the tumour cells themselves secrete a hormone-like substance known as growth factor, which switches on the gene in the stromal cells.

Scientists already know of compounds that block other metalloproteinases, and one may exist to block stromelysin-3. The enzyme occurs in the uterus and the placenta, and also in the foetus, where tissue is remodelled. This remodelling is tightly controlled by something, says Chambon. The enzyme is a possible target for therapy. Another approach would be to make antibodies to stromelysin, then "load" them with a toxin targeted at the stromal cells. The American company Oncogene is in contact with Chambon. (Source: New Scientist, 22-29 December 1990)

"Switching off" cancer cells

Scientists in Australia say that they have for the first time reversed the growth of cancer cells by "switching off" the gene that caused the disease, Reuter reports.

A genetic engineering process developed by Hiroto Naora of the Australian National University in Canberra quickly reversed the growth of fibrosarcoma cancer in a laboratory culture dish. The cancer cells were restored to a benign state.

Naora's team introduced a gene close to a cancer causing oncogene and used a biochemical trigger to stimulate it. The new gene inactivated the rogue oncogene.

The Canberra scientists had also switched off oncogenes in laboratory mice, which have a genetic arrangement similar to humans, Naora said. There was no reason why the process could not be used to control human cancers.

In theory the trigger could be inserted in a naturally occurring cancer and activated, but this would be difficult. An easier process would be to find naturally occurring trigger genes rather than insert one near the oncogene. Clinical application may still be 10 years away. Contact: Australian National University: Australia, 6 249 5111. (Source: Venture India, July/August 1990)

Isolating the chemicals that cause cancer

A new technique has the potential to lay bare the history of a cell's exposure to cancer-causing chemicals, or carcinogens. Once such chemicals are identified, it is possible to stop people coming into contact with them, the first step in any programme to prevent a particular sort of cancer.

David Phillips and his colleagues at London's Institute of Cancer Research have been developing the technique, called "postlabelling". It uses radioactive phosphorus to identify chemicals that attach themselves to a cell's DNA, causing cancer.

Postlabelling exploits the fact that naturally occurring phosphorus in the nucleotides, that make up DNA, can be replaced, or "postlabelled", by radioactive phosphorus. Phillips and his colleagues break down the DNA into its individual nucleotides, and then run them on an electrophoresis gel.

Nucleotides that have carcinogens attached have physical properties different to those of nucleotides by themselves, and are characteristic of that carcinogen. They therefore appear as separate spots on the gel. Each spot's position indicates the nature of the carcinogen, revealed when the radioactive phosphorus acts on a photographic film.

Postlabelling is so sensitive that it can identify just one molecule of a carcinogen in the genome and it can achieve this feat with a microscopic quantity of DNA, obtained from only a few cells.

Researchers realized as long ago as the 1960s that chemical carcinogens cause cancer by sticking onto the cell's DNA, largely as a result of work by Peter Brookes and Phil Lawley at the Institute of Cancer Research. Now, using postlabelling, it is possible to discover which chemicals have become attached to the DNA of humans which ones could cause cancer. (Source: New Scientist, 1 December 1990)

Gene therapy to treat cancer

The United States Federal Drug Agency has given NIH scientists the go-ahead to begin the first study using human gene therapy to treat advanced melanoma,

the most deadly form of skin cancer. Patients will receive transfusions of special cancer-killing cells, tumour-infiltrating lymphocytes or TIL, that have been altered by insertion of the human gene for tumour necrosis factor. The scientists grow the gene-altered TIL in the laboratory for four to six weeks before returning them to the patient by transfusion. The first patients are expected to be treated within the next six weeks. FDA has given approval for treating up to 50 melanoma patients. The study is being conducted by Steven. A. Rosenberg, the lead scientist, and R. Michael Blaese of the National Cancer Institute; W. French Anderson of the National Health, Lung and Blood Institute; and others. Rosenberg says, "Ultimately this new technique could lead to the use of gene therapy to correct or ameliorate a wide range of diseases, including cancers other than melanoma; heart disease; diabetes; and other inherited disorders such as haemophilia and cystic fibrosis." (Reprinted with permission from Chemical and Engineering News, p. 7, 26 November 1990. Copyright (1990) by the American Chemical Society)

IL-4 may inhibit organ rejection

Immunex (Seattle, WA) scientists have presented pre-clinical results showing that soluble interleukin-4 (IL-4) may also inhibit organ rejection at doses 2,000 times lower than antibody treatments.

The company disclosed that it has developed a "fusion molecule", produced in yeast, that combines IL-3 and granulocyte macrophage-colony stimulating factor (GM-CSF). Immunex believes the combination molecule may prove more efficacious than either compound alone - or when they are administered together - because certain receptors on the surface of white blood T-cells bind to both IL-3 and GM-CSF. The fusion molecule thus could target several receptors at once, better signalling blood cell growth. (Source: Bio/Technology, Vol. 8, October 1990)

Possible new treatment for auto-immune diseases

A compound from an Egyptian plant could be useful for treating auto-immune diseases. The drug, 8-methoxypsoralen (8-MOP), is produced by a plant that the ancient Egyptians knew made a person more sensitive to sunlight. The compound is now being tested for incapacitating white blood cells that attack the body's own tissues. The patient takes the drug and his blood is then exposed to UV light, a technique known as photopheresis. Although researchers have been researching this compound for eight years, they have not publicized it, since its mechanism is still unknown. 8-MOP could be useful in treating rheumatoid arthritis, multiple sclerosis, lupus and organ transplants. Each clone of T-cells specializes in attacking a foreign invader. 8-MOP attacks the cells as they divide by binding to the DNA of dividing cells. The dying T-cells are then identified as foreign and are attacked by other components of the immune system. This in effect vaccinates the patient against that class of T-cells. The drug has been adequately tested only on two diseases, but has shown great promise in preliminary tests on a number of auto-immune diseases. The technique is being pioneered by Dr. Richard Edelson of Yale University School of Medicine. Edelson first tried 8-MOP on a patient with cutaneous T-cell lymphoma, which did not respond to chemotherapy. Leukapheresis, the second-line treatment, is only temporary, since cancerous cells reappear. 8-MOP with UV light was tried to bolster the effects of leukapheresis. Although Edelson first tried the treatment simply to see if it would harm his patient, the patient became dramatically better after only two treatments, and is now disease-free. Of the next 37 patients, 27 responded. The treatment is also effective against scleroderma. Rheumatoid arthritis and multiple sclerosis might also respond. (Extracted from New York Times, 23 October 1990)

Human antibodies cloned in E. coli

Stratagene Corp. has cloned and expressed the gene for a human antibody to the tetanus toxin, bypassing mice and the hybridoma technology usually used to produce monoclonal antibodies. Company scientist Joseph A. Sorge and Scripps Institute researcher Richard A. Lerner estimated that their human immuno-expression library contains 20,000 clones with high affinity to the toxin. Stratagene's subsidiary, Stratacyte Corp., is offering non-exclusive licences to the technology, while its parent company is interested in creating joint ventures to develop its commercial products. (Source: McGraw-Hill's Biotechnology Newswatch, 5 November 1990)

Immunex fuses GM-CSF and IL-3

Biotechnology firm Immunex (Seattle) has genetically engineered a fusion molecule composed of granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-3, two promising therapeutic cytokines already in US and European clinical trials. Laboratory tests show that the fusion molecule, produced in yeast, is 10 times more active than a simple combination of the two compounds in promoting bone marrow cell growth, says Immunex. Both GM-CSF, which promotes white blood cell growth, and IL-3, which stimulates early blood cell development, are being developed to alleviate blood cell damage in cancer patients treated with radiation or chemotherapy. Clinical trials of the fusion molecule could begin in 1991, says Immunex. (Source: Chemical Week, 12 September 1990)

Cardiologists court r-DNA repairs of the heart

Borrowing from the work of their colleagues in oncology, immunology and haematology, cardiologists are taking the first, tentative steps in applying genetic engineering to treat conditions that lead to cardiovascular disease. So say researchers Gary J. and Elizabeth G. Nabel of the Howard Hughes Medical Institute, University of Michigan (UM), Ann Arbor, and Stephen E. Epstein, of the National Heart, Lung and Blood Institute (NHLBI), Bethesda, Md.

Epstein and Nabel delivered papers on the potential of recombinant DNA in cardiovascular disease at the 63rd Scientific Sessions of the American Heart Association (AHA). Nabel reported on a localized form of gene transfer useful in studying cardiac disorders. Epstein told of preliminary results on the use of chimeric toxins - a technique he borrowed from oncology - in treatment of restenosis, the uncontrolled growth of vascular smooth muscle cells that often follows balloon angioplasty.

For the past two years, Nabel has been searching for a way to insert and achieve expression of genes in focal arterial sites in the

vasculature. These genes alter the biological processes in the area. In the long term, she hopes to apply the method to reduce atherosclerotic lesions.

Nabel and her co-workers devised a technique, in which she delivers the altered genes via a catheter and retroviral vectors. She predicts that it will have several therapeutic, as well as research, applications, ranging from treatment of atherosclerosis, to prevention of heart transplant rejection.

Nabel has so far transferred marker genes into endothelial cells in the arteries of live pigs and observed expression of genes only at the implantation site. In her subsequent study, presented at the AHA meeting, she stimulated a localized inflammatory response by inserting a gene for antigens that cause transplant rejection and chronic inflammatory disease.

Molecular biologist Gary Nabel, a co-author of the paper, is also exploring therapeutic regimens based on localized gene therapy to treat conditions involving abnormal cell growth - restenosis and cancer.

NHLBI's Epstein decided, about a year ago, that a "major future direction would be to combine the power of molecular biology with the traditional physiology/pharmacology approaches". Together with Ira Pastan of the National Cancer Institute (NCI), whose laboratory has been experimenting with "chimeric toxins" specifically pinpointed to cancer cells, and molecular biologist Clay Siegall, Epstein also targeted restenosis. "The response of the vascular wall to these procedures is often the overproliferation of smooth muscle cells. Epstein observed that these rapidly proliferating cells expressed more growth factor receptors than did normal cells. These receptors provided specific targets for a chimeric toxin.

He started with Pseudomonas exotoxin, a highly potent toxin of 66,000 daltons, arranged in three domains - the first controlling cell recognition, the second, translocation and the third, toxicity. In earlier experiments, NCI's Pastan used genetic engineering to delete the first domain. The remaining 40,000 dalton protein (PE40), maintains its toxicity but is unable to recognize receptors, and therefore, cannot enter cells.

Epstein then fused PE40 to a growth factor. This fused, or chimeric, toxin binds to overabundant growth factor receptors, killing only the abnormal smooth muscle cells. In in vitro experiments, the chimeric toxins destroyed these over-proliferating cells. He adds that he is in the process of moving into in vivo studies. (Source: McGraw-Hill's Biotechnology Newswatch, 19 November 1990)

Engineering antibodies

Monoclonal antibodies are a biotechnologist's best friend. Each one - and the immune system can make millions of different sorts - will recognize one specific molecule. They can be used to find a particular type of cell, by recognizing its surface molecules, or to pick a protein out of a complex biological soup. The trouble is that they are laborious to produce artificially. In a paper published in Nature, scientists at Cambridge Antibody Technology, together with the Medical Research Council's molecular-biology laboratory in Cambridge, announced an easier way to make such friends. They have found a clever method of manufacturing highly specific antibodies.

The Cambridge group started off with the genes that describe the various antibodies that recognize a protein called lysozyme, a readily available protein found in tears. Antibody genes differ in "variable regions", which describe the part of the antibody that actually sticks to the target molecule - the business end. By mixing and matching the variable regions of these genes it is possible to create lots of different sticking points, which recognize slightly different aspects of the lysozyme molecule.

In a spirit of not-exactly-idle curiosity, the researchers made a hybrid gene: it is made in part from the variable region of a gene describing an antibody that recognizes lysozyme, and in part from a gene found in fd, a virus that preys on bacteria. The viral gene they used was one that describes a protein making up part of the virus's outer coat. To the researchers' delight, when they infected bacteria with viruses containing the hybrid gene, the bacteria started producing mutant virus particles that had antibody fragments on their surfaces. These antibody-bearing viruses were sorted out from their brethren by being passed down a column stuffed with lysozyme. The antibody fragments stuck to the lysozyme; one antibody-bearing virus can thus be plucked out from a million others.

In the next few months, the group hopes to use its technique to make a suite of different lysozyme-recognizing viruses, using genes with different variable regions. The different viruses can be told apart by exposing them to sub-units of lysozyme. Those best at recognizing the protein can easily be found. It is this that gives the new technique its advantage. The original technique for making monoclonal antibodies required removing a lot of immune cells from a mouse, making them immortal by mixing them with tumour cells, and then testing the antibodies from each new set of cells to find out what they did. Using the new technique, when scientists find an antibody-bearing virus doing something useful, the genes that describe the antibody part of it are right to hand, all wrapped up in a neat little viral package. In theory, it is a small matter to then put the genes into cells that will churn out antibody fragments.

The Cambridge team believes it has a powerful new tool, even though only a few antibody genes have yet been cloned and fully described. A whole library of antibody genes - from mice or even humans - could be incorporated into viruses. The viruses could then be screened against all sorts of targets. Such screening would allow the library to be catalogued and arranged. Or scientists could invent new antibodies of their own, based on the gene fragments already studied, and improve, perhaps, on what nature provides. (Source: The Economist, 8 December 1990)

Quick fix found for cystic fibrosis gene

A year after locating the minute genetic fault that causes cystic fibrosis, scientists in the US have succeeded in replacing the faulty gene in human cells in a test tube. The altered cells functioned properly, leading the researchers to predict that gene therapy may soon treat the disease.

The success with the cystic fibrosis gene comes less than a week after the first gene therapy was performed on a human, a four-year-old girl with an enzyme deficiency that cripples the immune system. James Wilson of the University of Michigan, whose research partner, Francis Collins, helped discover the genetic defect last year and his team raced to prove their method before that of another team composed of scientists from the University of Iowa, Tufts University and a company called Genzyme, both based in Massachusetts. Both groups succeeded within one week of each other.

Cystic fibrosis is the most common inherited disease among white people in the US, affecting about 30,000 people at any one time. It is caused by the failure of cells in the lung, sweat glands, intestine and pancreas to get rid of chloride ions. Normally, these leave the membrane of the cells through ion channels. In people with cystic fibrosis, these channels are blocked. Thick mucus, which builds up in the lungs in particular, provides a rich breeding ground for bacteria and other micro-organisms. Few people with cystic fibrosis live beyond their twenties.

Last year, Collins and Lap-Chee Tsui of the University of Toronto tracked the cystic fibrosis defect to a gene consisting of about 250,000 base pairs, the chemical units of DNA. The flaw was a deletion of just three of these base pairs. As soon as the discovery was made, Wilson started trying to replace the defective gene with a healthy one. He removed cells from the pancreas of a person with cystic fibrosis. He used a retrovirus to ferry the gene into the cells. Once inside, the normal gene began producing a protein cystic fibrosis transmembrane regulator in the normal fashion. This opened the chloride ion channels.

The group at Iowa and Massachusetts, led by Michael Welsh, used another virus, Vaccinia, which is commonly used to vaccinate humans against disease. They infected cells taken from the air passages of people with cystic fibrosis and achieved the same result as Wilson's team. Welsh's group reinforced their findings by inserting a faulty gene into the same type of cells. The chloride channels in these cells failed to open.

Work on human air passages are the route to the next step: trying the technique directly on animals and humans. The first human experiment in gene therapy, reported last week, used a technique known as replacement gene therapy. This involves removing faulty cells, adding a gene and then replacing the cells.

This technique is not feasible with people with cystic fibrosis because it would involve altering a large number of cells, Wilson says. His team hopes instead to add healthy genes directly to affected cells, either using viruses or a new complex of DNA and protein with which he is experimenting. People could inhale healthy genes regularly as an aerosol spray, he suggests. Such thereapies require lengthy study, Wilson warns, but "this is where the research should go".

In the second major advance in cystic fibrosis research in two months, researchers have discovered that the molecular mechanism of most cystic fibrosis involves the deficiency of a specific protein on surfaces of affected cells. In September, two independent scientific groups corrected cystic fibrosis cells, which bear a defective gene, by adding a normal gene to the cells. Now, researchers from one of those groups also show that the product of the defective gene - a protein called cystic fibrosis transmembrane conductance regulator (CFTR) - does not reach its proper destination on the cell surface because it is incompletely processed within the cell. In the study, Alan E. Smith and his team at Genzyme Corp., Framingham, Mass., induced specific mutations in the cystic fibrosis gene and characterized the proteins encoded by these alterations. Normally, CFTR on the cell membrane helps transport chloride ion out of the cell. However, study revealed that several gene variants produce immature protein that is not transported to the cell membrane. The absence of mature protein on the cell surface results in a change in chloride transport that may cause the characteristic mucus build-up. According to Smith, the results demonstrate "that cystic fibrosis is a protein deficiency disorder ... rather than a defect in protein function". (Source: New Scientist, 29 September 1990 and Chemical Engineering News, 19 November 1990)

Research on animal genes

Immunex finds MGF gene

Scientists at Immunex (Seattle) report discovering and cloning the gene for mast-cell growth factor (MGF), a protein associated with anaemia and pigmentation defects in mice. MGF is one of a number of colony-stimulating factors (CSFs) under development that have the ability to promote production of various blood cells in the body. MGF, says Immunex, acts early in the blood-cell development process before the cells differentiate and could have a wider range of effects than other CSFs. Immunex says the MGF work could lead to treatments for anaemia, as well as blood conditions associated with bone-marrow failure and chemotherapy. (Source: Chemical Week, 24 October 1990)

Bovine growth hormone produced in silkworm

Nihon Nosan Kogyo K.K. has succeeded in introducing a cloned bovine growth hormone gene into a silkworm virus and then infecting the silkworm. The researchers were able to obtain a production output of 500 µg of growth hormone per ml of silkworm body fluid. (Source: ABA Bulletin, Vol. 5, No. 6, December 1990)

Pig transplants cure diabetic mice

Diabetic mice have been cured by transplants from pigs, according to researchers at the Australian National University. If such a feat were possible in humans, diabetes might be curable. The transplants involved pancreatic pro-islets from pig foetuses. The transplants were tolerated by recipient mice because their helper T-lymphocytes were de-activated by monoclonal antibody to the CD4 antigen expressed on helper T-cell surfaces. After 12 days of anti-CD4 injections, helper T-cells were virtually wiped out. They were then allowed to return, and reached normal levels within about 26 weeks. Meanwhile, the islet cells were able to grow and mature. The restored T-cells did not attack the cells or any pig cells subsequently introduced with the same major histocompatibility complex antigens as the graft. Some mice have lived with the graft for 200 days without any sign of

diabetes. The treatment does not work in about 40 per cent of cases, however, for reasons that are not clear. Perhaps the anti-T-cell treatment needs to be more aggressive. (Extracted from New Scientist, 22 September 1990)

Proteins strengthen sea urchin skeletons

Proteins in the calcite crystals of sea urchin skeletons are responsible for the unusually high fracture resistance of these crystals, according to Amir Berman of the Weizmann Institute of Science, Rehovot, Israel, and co-workers there and at Brookhaven National Laboratory, Upton, New York. The work reflects widespread current interest in mimicking nature to make new materials. Calcite crystals in sea urchin skeletons are much more resistant to fracture than pure calcite crystals, which cleave easily along well-developed planes. The possibility that this enhanced fracture resistance is caused by the presence of glycoproteins in the crystals was first suggested in a scientific paper published in 1916. Now, Berman and colleagues confirm this concept. Based on X-ray diffraction and synchrotron radiation studies, they propose that the ability of the glycoproteins to stabilize discontinuities in the crystal helps interfere with the propagation of smooth fractures. "If proven general", they say, "this principle may well have broader application to the materials sciences as a means of creating new single-crystalline composites intercalated by specifically absorbed intracrystalline polymers". (Reprinted with permission from Chemical and Engineering News, p. 3, 5 November 1990. Copyright (1990) by the American Chemical Society)

Deep-sea fishing dries up the gene pool

Commercial fishing can seriously reduce the genetic diversity of wild fish, according to researchers in New Zealand. Their study of the orange roughy (Hoplostethus atlanticus) shows that heavy fishing can cause noticeable effects in just 10 years. It has long been known that farmed fish, such as salmon and trout, tend to become more like each other as variability is bred out of them but, until now, there has been little evidence that fishing of wild stocks might have a similar effect.

Peter Smith and his colleagues at the New Zealand Ministry of Agriculture and Fisheries Research Centre in Wellington studied the genetic variation in several stocks of the orange roughy. They believe that fishing leaves an impoverished gene pool, because it removes the largest and older fish, which are the most genetically variable.

The orange roughy is a deep-water fish which is widespread in the North Atlantic and Indian Oceans and the southwest Pacific. It was once thought rare, but the discovery of large spawning aggregations around New Zealand led to the rapid development of a commercial fishery in the early 1980s.

The orange roughy grows relatively slowly. It may live for 50 years but reaches maturity late, at around 20 years.

Usually, a wide species becomes less varied genetically when a catastrophe of some kind dramatically reduces the size of a population leading to an evolutionary "bottleneck". The animals that come through the bottleneck are the source of all the genes of their descendants, and so those descendants are very similar genetically.

Likewise, populations that have been isolated for a long time suffer from the "founder effect", and inherit genes from a small number of individuals. When a population of wild animals is exploited until it is nearly extinct, this has the same effect.

Smith and his colleagues studied the genetic variation in three stocks of orange roughy - in 1982 when the fishery was just beginning, and again in 1988. They compared a number of enzymes that are the products of known genes. A fish has two copies of the gene for each enzyme. If the two versions, or alleles, are identidal, the fish is homozygous for that enzyme; if they differ, it is heterozygous - in other words, it shows genetic variation. The loss of genetic variation manifests itself as an increase in the level of homozygosity - more and more of the genes in the population are the same.

On each fishing ground, Smith and his colleagues found a marked decrease in heterozygosity from 1983 to 1988. This suggests that there has been a significant genetic change in the orange roughy populations around New Zealand in less than a decade.

The researchers rule out any other explanations for the change. It seems unlikely, they say, that they sampled different populations in 1983 and 1988, because the same trend shows up in all three sampling areas, which are widely separated geographically. They also dismiss the idea that they may have sampled fish of different year classes, which might have a different genetic constitution. Because orange roughy are so long-lived, they sampled animals of many ages on both occasions. Nor, they claim, did some natural catastrophe dispose of all the more varied individuals. Homozygous animals are generally at greater risk from natural disasters, such as disease.

The explanation of how fishing removes the most genetically varied individuals is bound up with the slow growth of the fish. According to many genetic studies, heterozygous individuals grow faster and often larger, so that in an unfished population the biggest fish will be the most varied genetically. In many species, the oldest individuals return to their spawning grounds first and stay longest - increasing their chances of being caught. The conclusion is that fishing selectively removes the largest and most genetically varied individuals, leaving the less variable individuals to breed. (Source: New Scientist, 1 December 1990)

Research on plant genes

Jumping genes confound German scientists

In the summer of 1990, scientists in Germany carried out the country's first large-scale release of a genetically modified organism. They planted a field of petunias in Cologne, but it has become clear that the results of the experiment are completely different from those the scientists expected. According to Heinz Saedler, a scientist at the Max Planck Institute for Plant Breeding in Cologne, this is just one more example of the complexity of nature and hence the need for experiments to be done.

Saedler and his colleagues set out to trap a so-called "jumping gene" in petunias. By doing this, they hoped to study how such genes function in an easily manipulated plant. The petunias were

implanted with a pigment gene from maize, which turned the flowers salmon red. The maize gene was linked to a "promoter" sequence from the cauliflower mosaic virus. This promoter turned on the gene in all regions of the plant.

Saedler and his colleagues grew the petunias in a greenhouse. They predicted that, as the petunias blossomed, some of the flowers - a very small number - would turn wholly or partly white. This would happen as the petunias' jumping genes jumped into the middle of the maize gene, and prevented it from being expressed.

White would, therefore, signal that a jumping gene was present in the maize gene. The scientists could then easily isolate it from the plant's DNA. In this way, the maize gene would become a trap for the jumping gene, says Saedler. Once trapped, the jumping gene could be purified in relatively large quantities for further study.

Saedler did indeed see very few white petunias in the greenhouse. But because the event was expected to be so rare, obtaining enough white flowers to study properly required large numbers of plants. The only option was to plant the petunias outside.

The experiment was authorized by the Government before Germany's gene law came into effect last July. In May, the Max Planck scientists planted 30,000 genetically modified petunias over an area of 5,000 square metres.

Most of the plants bore red flowers, but a few did have white ones. Some were completely white, which would be expected if the red pigment gene had been turned off very early in the seed's development. Some plants had both red flowers and white ones. Some had variegated flowers, as if the "jumping" gene had never affected some cells, or departed from some pigment genes and allowed the red pigment to reassert itself.

"All this was exactly what we expected", says Saedler. "But much to our surprise, we found that the frequency (of white colouring) was about 10 times too high". About 6 per cent of the flowers were white, and 0.1 per cent were variegated.

Then, during a heatwave in July and August, all the flowers turned white. Such bleaching is normal in petunias under high-intensity light. But after the heatwave, instead of reverting to the same frequencies of red and white, the petunias produced white and variegated flowers about eight times as often as they had previously.

The team has isloated DNA from 66 of the white-flowered plants, and has so far analysed 17 of them. In none of them is the white colour due to the presence of a jumping gene. When petunia DNA is chopped up with "restriction" enzymes, which cut DNA in specific places, the sizes of the resulting fragments reveal what has happended to the genes. The scientists found no large fragments that would reveal the presence of a jumping gene within the maize gene.

Instead, DNA from petunias with white flowers responded to the restriction enzymes in a way that showed the promoter of the gene had a methyl group (containing a single carbon atom) added to it. This is what turned off the red pigment. In variegated flowers, genes in some cells were either never methylated, or become demethylated, allowing the red pigment to be expressed.

A plant may yet be found in which the white colour was due to a jumping gene, says Saedler, but the result is now useless. White flowers were supposed to signal the presence of a jumping gene in the maize gene. If other things turn off the pigment gene, the signal is not specific for jumping genes, and the point of the screening system is lost.

Instead, Saedler now wants to know what causes methylation. The team has applied under Germany's new gene law to plant 20,000 petunias in a further trial in 1991, to test 160 different combinations of factors that may be important. They will compare plants that have the maize gene at different positions within the genome, and that have different numbers of copies of the gene. They will also test the effects of different day lengths, positions of flowers, and temperatures by comparing plants grown in growth chambers, the greenhouse, and the field. (Source: New Scientist, 15 December 1990)

"Restorer gene" reverses sterility, completes r-DNA plant hybridization

A newly discovered gene that restores fertility to sterile plants is the final component of a quicker, easier hybridization system, says Jan Leemans, research director at Plant Genetic Systems N.V., (PGS) Ghent, Belgium. Leemans announced the discovery of this "restorer gene", as well as the results of the first field-trials testing the initial genetically engineered male-sterility components at the Ag Technology '90 conference.

The field-tests were conducted in Belgium and France on spring rapeseed. Oilseed rape - which he estimates to be a $90-million seed market in Europe - will be the firm's first commerical target. Commonly known as canola, this crop has resisted conventional cross-pollination techniques, as have wheat and other small grains. Leemans declares that PGS's new system "can be applied to any crop". In April, PGS contracted with Japan Tobacco, Inc., Tokyo, to develop a better variety of hybrid rice.

Three years ago, PGS saw the opportunity to use genetic engineering to improve the painstaking task of producing the hardier, higher yielding hybrid seeds. The firm identified three essential components - development and maintenance of male sterility, and a way to restore fertility, so the plants will set seed.

PGS reported the discovery of the sterility gene - a single dominant gene that prevents pollen production - just one year ago.

"The next challenge was how to maintain the male sterile material", he recalls. In classicial breeding, it is maintained by crossing the plants with special lines that transfer the trait. "In our test, the simple solution was to link the gene for male sterility to a gene for herbicide tolerance. When we cross these male sterile plants with any other plants, the offspring segregate - fertile and herbicide-sensitive and sterile and herbicide-tolerant. A single herbicide application will eliminate the undesirable plants".

For seed crops, restoring fertility is the conclusive, critical step, Leemans says. "Seed producers need a final, fully fertile, hybrid. So we must be able to reverse the process of male sterility". PGS developed the restorer from a naturally occurring inhibitor of the gene used to induce sterility in the first place. Leemans says that the two genes neutralize one another, and have no effect on other agronomic factors. (Source: McGraw-Hill's Biotechnology Newswatch, 1 October 1990)

Foreign DNA inserted into plant chloroplasts

Scientists at Rutgers University, New Brunswick, NJ, have inserted foreign DNA into chloroplasts of tobacco plants, the first such insertion in a multi-celled plant.

Similar modification of the simpler chloroplasts of green algae was achieved three years ago by Botany Professor John Boynton and colleagues at Duke University.

The Rutgers work may lead to improved understanding of the regulation of gene expression in chloroplasts. It may also boost knowledge of how genes in chloroplasts interact with those in cell nuclei and how chloroplasts are involved in metabolic and developmental processes.

On a commercial level, inserting herbicide- and pest-resistant DNA into chloroplast genes may be an attractive alternative to genetic modification of cell nuclei. This is because plant pollen cells contain no chloroplasts. Thus, chances for escape of modified genes into the environment through spread of pollen would be minimized. (Abstracted with permission from Chemical and Engineering News, p. 6, 12 November 1990. Copyright (1990) by the American Chemical Society)

Splice of life for ailing vineyards

In the future, you may be drinking wine made from genetically engineered grapes. Californian researchers announced they had succeeded in transferring foreign genes into vine rootstock with the aim of introducing resistance to pests and disease and regulating growth and development. But the breakthrough is likely to herald enormous confusion in Europe where the deeply entrenched traditions of the wine-growing community have spawned a strict regime for regulating the sale of wine.

Michael Mullins and Archie Tang of the Department of Viticulture and Enology at the Univeristy of California, Davis, and Daniel Facciotti of the biotechnology company Calgene, also in Davis, demonstrated a means for transferring genes into a rootstock called Rupestris St. George (Vitis rupestris) and into two well-known varieties of grapevine, cabernet sauvignon and chardonnay. The genes they introduced were simply markers to demonstrate that gene transfer had occurred.

In Europe, however, the use of traditional cultivars (long-established varieties of vine) is required by law.

The wine regime rules in Europe give a definition of wine as "a natural product of fresh grapes". It also lists vine varieties acceptable within the Community.

The Californian researchers recognize the hurdles a new wine would face. Despite the prospect of opposition to new varieties, work on the application of biotechnology to grapevines is under way in Europe, explained Armin Gemrich, a leading viticulture researcher at the technical college in Heilbronn near Stuttgart. He said that the most advanced work is at the Federal Breeding Station in Teileweilerhof near Landau, though no transgenic grapevines had been produced yet.

An important advance would be a genetic implant that confers resistance to the American root louse, phylloxera, which was introduced accidentally into Europe at the turn of the century and devastated vineyards. Since then, viticulturists have had to graft resistant American rootstock, including St. George, to almost all species of grapevine.

The Californian researchers have used a standard technique for inserting the foreign genes. They used an organism called Agrobacterium tumefaciens as a "Trojan horse" to insert the foreign genetic material into the new hosts. A. tumefaciens is a common soil-dwelling bacterium that infects roots of plants, causing tumours to grew on the tendrils.

Biologists found in the 1980s that, in nature, the bacterium is able to integrate some of its own DNA into that of its host. Since then, genetic engineers have exploited this property, using disarmed A. tumefaciens to shuttle new genetic material into plants.

The researchers in California inserted two genes into the grapevine stock. One gene carried instructions for producing a blue chemical called B-glucuronidase which, if produced in the new host, would demonstrate visually that the gene had been implanted.

The other gene confers resistance against kanamycin, an antibiotic. Any plants not containing the gene would die if exposed to kanamycin, so only the plants with the successfully implanted gene would survive.

The researchers applied the gene-carrying bacterium to so-called "explants", parts of the embryonic plant. They achieved most success with the rootstock, which went on to form buds carrying the new genes. From these, they succeeded in making a whole plant. With grapevine, they did create buds with implanted genes but have not managed to grow a whole plant. (Source: New Scientist, 22-29 December 1990)

Research on viral genes

HIV target revealed

Two US research teams have independently elucidated a partial atomic structure for CD4, the human cell surface molecule targeted by HIV. The new work precisely defines two of CD4's four glycoprotein domains.

Armed with a map of the protein's structure, researchers may gain a better understanding of the infection process, and develop improved therapies for AIDS.

Both Wayne Hendrickson's team at Columbia University and Stephen Harrison's group at Harvard

identified two immunoglobin-like domains, connected by a continuous β-strand. Domain 2 has an unusual disulphide bond, in the form of an intra-sheet bridge.

The initial event in infection is binding of the viral protein gp120 to CD4 on the cell surface, and the researchers have analysed the amino acid sequences involved in this interaction. Several companies, including SmithKline Beecham, have been working on drugs that would block HIV binding, but so far clinical trials have been less than encouraging.

Several SmithKline scientists contributed to the work at Columbia. According to a company spokeswoman, SmithKline has abandoned work on soluble CD4 proteins, but is now seeking small CD4 analogues which would work in a similar fashion, acting as a "decoy" for the virus.

Genentech announced that a human pilot study of gp120 as a potential treatment for HIV infection has been initiated at the Walter Reed Army Institute of Research in Washington, DC. In June, Genentech scientists reported that inoculation with a subunit gp120 vaccine protected chimpanzees from HIV infection.

Researchers at Walter Reed will now try to determine if post-exposure immunisation with recombinant gp120 can boost the immune systems of HIV-positive patients. The study will involve 55 patients, and initial results are expected within about 10 months.

Genentech says it is also considering a separate human pilot study to test recombinant gp120 as a potential vaccine to protect uninfected individuals. (Source: Chemistry & Industry, 17 December 1990)

New anti-HIV agent is a diazepinone

A new chemical structure that strongly inhibits the reverse transcriptase of human immunodeficiency virus-1 has been discovered by researchers at Boehringer Ingelheim Pharmaceuticals in Ridgefield, Connecticut. Most of the agents known to inhibit this key enzyme in the AIDS infection process are nucleoside analogs. These include zidovudine (3'-azido-3'-deoxythymidine or AZT), which is FDA-approved, and 2',3'-dideoxyinosine (DDI), which is still experimental. The new drug, code-named BI-RG-587, is a cyclopropane-substituted diazepinone with a pyridine ring fused on either side. Because it is chemically different from AZT and DDI, it may prove to be clinically effective where AZT and DDI are not or where they have lost their efficacy. The researchers hope to avoid the toxic side-effects observed in patients taking nucleoside analogs. In test-tube experiments, BI-RG-587 acts as a non-competitive enzyme inhibitor with "exquisite specificity" against HIV-1 reverse transcriptase and "extremely low" cytotoxicity in uninfected human cells, according to Vincent J. Merluzzi and co-workers. Early animal tests on this antiviral agent also are encouraging, they say. (Reprinted with permission from Chemical and Engineering News, p. 17, 10 December 1990. Copyright (1990) by the American Chemical Society)

HIV enters brain cells through surface receptor

AIDS patients often suffer a form of dementia, losing their balance and memory, and having severe behavioural problems. Until now, this has mystified medical scientists. But now it seems that dementia may come about when HIV enters brain cells through a newly discovered surface receptor.

Uusally, HIV attacks the lymphocytes of the immune system, binding specifically to receptors known as CD4 molecules. Neurons and other brain cells are not known to possess these receptors and so would seem to be safe.

But Yaffa Mizrachi and her colleagues at St Luke's-Roosevelt Hospital believe they have located a receptor which allows HIV to enter cells in the brain. The receptor is on the membrane of neurons and glial cells, the support cells of the brain. Mizrachi believes that HIV uses a protein on its outer coat, known as gp120. In the test tube, she was able to successfully infect brain cells with gp120 via a receptor other than CD4. Once it has attached itself to the cells, the viral protein entered by fusing with the membrane.

Mizrachi is now trying to characterize the receptor that allows entry, and locate the gene that codes for it. She believes it might then be possible to devise a treatment for AIDS patients with dementia that would block these receptors, leaving the virus with no place to link up.

But researchers are still puzzled about how the virus harms brain cells once it enters them. According to David Volsky at St Luke's, once HIV is integrated into brain cells, it does not replicate well enough to do the sort of damage it does in the cells of the immune system.

Stuart Lipton at Harvard University has suggested how this might work. He has discovered that gp120 appears to make nerve cells more sensitive to glutamate, which they use for communication. Too much glutamate, which occurs in cases of stroke or trauma, is believed to allow calcium in cells to reach harmful levels. Over-sensitivity to glutamate could cause the motor abnormalities and reasoning disabilities associated with AIDS dementia, says Lipton. (Source: New Scientist, 17 November 1990)

AIDS vaccine "may not work" in Africa

Some of the most promising candidates for an AIDS vaccine could be useless outside the West. Evidence is growing that many of the strains of HIV found in Africa are different from the strain on which most research has focused. The differences could make many of today's potential vaccines and therapies ineffective against the African strains.

Scientists at the Fifth International Conference on AIDS in Africa, called for research into African strains of the virus. Only then could appropriate vaccines be developed, they said. The call came before Zaire's leading AIDS researchers met Dan Hoth, from the National Institutes of Health in the US, to discuss possible trials of a vaccine in Zaire.

The trials would be the first of their kind: they would test not simply whether the virus was safe, but whether it worked in preventing the spread of HIV or slowing the progress of the disease. It is not certain whether the people to receive a vaccine would be pregnant women infected with the virus, prostitutes or couples where one partner is infected. For the pregnant woman, the aim would be

to prevent transmission of the virus to the infant. Zaire's ethics committee has still to approve the trial. Vaccine trials have become controversial in Zaire after Daniel Zagury, a French researcher, inoculated himself and a group of Zairian volunteers with a vaccine based on a modified vaccinia virus. Secrecy has surrounded these trials and Zairean researchers are loath to comment on them.

New research presented at the conference shows that more and more differences are emerging between the strain of HIV isolated by Robert Gallo and Luc Montagnier, and strains of HIV-1 from Africa. An Ethiopian team said they had sequenced a group of strains from their country that had common characteristics. According to Seyom Ayehunie, leader of the team from Addis Ababa, these characteristics "set them apart from all other isolates of HIV-1 sequenced to date". Researchers from the Central African Republic also described strains that differed markedly from the classic strain.

Alash'le Abimiku, a virologist from the University of Jos in Nigeria, currently working at the National Institutes of Health, said vaccine researchers must work with strains found in Africa. She is trying to sequence isolates of HIV from Nigeria.

The strain of HIV from Africa that has received most attention, so far, is a highly virulent strain of HIV from Zaire. Scientists now based at a research unit in Marseilles, which is funded by the French Government, have been studying this strain of the virus since 1985.

The strain, known as NDK, is 10,000 times as effective at killing cells as the classic strain. Recently the team, led by Jean-Claud Chermann, discovered that the strain lacks a crucial feature that scientists believe may be HIV's Achilles heel. This is a peptide forming part of a loop known as V3 on the protein coat of the virus. The peptide is involved in infecting cells. Antibodies to the virus prevent the virus from infecting cells and many researchers are pinning their hopes on it as a potential vaccine. This peptide, called gpgr, is shared by the majority of western strains so it should in theory protect against most of them.

The discovery that NDK lacks the peptide raises fears that vaccines based on gpgr would not protect against this strain. Researchers focusing on gpgr in the West include Marc Girard of the Pasteur Institute and Scott Putney of Repligen in Cambridge, Massachusetts.

But the Marseilles team's latest discovery is equally alarming. Yvan Hirsch, a member of the Marseilles group, presented his findings at the conference. This research suggests that NDK can infect cells without entering via the usual receptor on the cell surface, CD4. He has found that, in the laboratory, the virus appears capable of infecting cells not normally vulnerable - epithelial cells from the lining of the lung and fibroblasts. The team has now identified the precise part of the genome that makes NDK so virulent. It is a part of the gene gag, which encodes HIV's protein coat. Until now, researchers had believed that the gene env controlled virulence. These latest discoveries could have implications for potential therapies for the virus, says Hirsch. (Source: New Scientist, 20 October 1990)

General

New technique suggests enormous increases in DNA-sequencing speed

Employing a new technique called "sequencing by hybridization" and 10 to 20 technicians, genome researchers using two machines will be able to sequence up to 100 million bases per day, a 5,000-fold increase over current methods. So say Rodoje Drmanac and Radomir Crkvenjakov of IMGGI in Belgrade, Yugoslavia, who described a new technique at the recent genome conference in San Diego. Their work is one of many efforts by scientists - responding to growing criticism of the Human Genome Project's $3-billion price tag - to find faster, cheaper ways to sequence the estimated 100,000 genes on the distinct human chromosomes.

At the Human Genome II conference, Drmanac and Crkvenjakov's sequencing by hybridization sparked the most interest. In this method, an array of oligonucleotide probes complementary to specific nucleotide sequences are permanently fixed to an identifiable glass bead or to a plate at known locations. Single DNA strands hybridize with the probes where the complementary bases match, revealing the presence of particular sequences at particular spots on the strand. The greater the length and number of probes on the array, the longer the DNA fragments that can be completely hybridized. For probes eight bases in length, for example, an array of 4^8 - over 65,000 probes - would account for all possible combinations and permutations of the four different bases.

A possible limitation to the technique, Drmanac pointed out, is that some of the sequences for which the probes are complementary may be repeated, suggesting that longer probes and/or shorter DNA segments have less probability of including repeating sequences.

Others investigating the hybridization technique include Bruce Jacobson at Oak Ridge National Laboratory, Tennessee, Ed Southern of Oxford University in the UK, and Andrei Mirzabekov of the Shemyatkin Institute in Moscow. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 December 1990)

Bound abzymes work in mixed solvents

Catalytic antibodies (abzymes) immobilized on inorganic supports can be used to catalyze reactions in mixed aqueous-organic solvents, say professor Kim D. Janda and colleagues at the Research Institute of Scripps Clinic, La Jolla. This is the first time that abzymes have been immobilized and the first use of abzymes in organic solvents (except in micelle systems), according to Janda. Lipase-like abzymes lose most of their activity when returned to aqueous solution after having been exposed to organic solvents. But when the abzymes are immobilized on glass beads, placed in organic solvents, and then returned to an aqueous environment, they retain the same activity and stereo-selectivity as in the free, unbound state. Furthermore, the immobilized abzymes can catalyze reactions directly in 40 per cent solutions of dipolar aprotic solvents such as dimethylsulphoxide. The ability to catalyze reactions in organic solvents is important because many potential substrates are insoluble in water. Immobilization also makes it possible to

wash and reconstitute abzymes that have lost their activity. The findings could therefore facilitate the design of immobilized abzyme reactors for industrial and pharmaceutical processing. (Reprinted with permission from Chemical and Engineering News, p. 28, 3 December 1990. Copyright (1990) by the American Chemical Society)

Drug delivery

Scientists in the US have developed a novel synthetic polymer for encapsulating sensitive biologically active entities. The new compound could find important uses in areas of medical science, such as drug delivery, where micro-encapsulation is finding increasing use.

Encapsulation using existing synthetic polymers requires heat or the use of organic solvents. The harsh treatment is often unsuitable when delicate entities, such as proteins or liposomes, are being encapsulated.

Natural polymers, such as the seaweed extract alginate, can be used as encapsulation materials. However, these natural polymers are often problematic as they have variable biocompatibility, and consistent reproduction of their properties can be difficult.

Smadar Cohen and his colleagues, from the Massachusetts Institute of Technology and Pennsylvania State Univeristy, have reported the development of a polyphosphazene that forms gel matrices by adding divalent cations in water at room temperature. These gels can encapsulate mamalian cells, liposomes and proteins.

The polymer poly[bis(carboxylatophenoxy) phosphazene] (PCPP), was found to be insoluble in acidic or neutral solvents, but soluble in basic solutions, such as sodium carbonate. The addition of calcium ions to PCPP resulted in rapid gelation. The authors suspect that salt bridges are formed between arboxylic groups of adjacent polymers, creating an ionically crosslinked matrix (Ca-PCPP). Microspheres could then be prepared using droplet-forming apparatus.

The polymer appears to have low toxicity. Cultured liver cells in contact with PCPP survived for at least five days. (Source: Chemistry & Industry, 19 November 1990)

Bioluminescent reporter for naphthalene

Inserting the luciferase genes associated with bioluminescence into a naphthalene catabolic plasmic yields an inducible bioluminescent plasmid that can be used as a reporter for environmental naphthalene. According to J.M.H. King and G.S. Sayler at the Univeristy of Tennessee's Centre for Environmental Biotechnology and co-workers there and at Oak Ridge National Laboratory, bacterial strains harbouring the recombinant plasmid produce enough light to serve as biosensors of naphthalene exposure and biodegradation. The researchers believe that bioluminescent bacterial strains could also be developed for other chemical agents and that such reporter organisms could be immobilized on fibre-optic probes for on-line monitoring and process control applications. The recombinant strains could also be useful as specific sensors for chemical agents in mixed-culture biological processes (such as waste treatment) and environmental systems (such as groundwater). (Reprinted with permission from Chemical and Engineering News, p. 22, 20 August 1990. Copyright (1990) by the American Chemical Society)

TS structure solved

Rational drug design company Agouron Pharmaceuticals (La Jolla, CA) has announced that its scientists have solved the three-dimensional structure of the enzyme thymidylate synthase (TS). As well, the company has revealed the atomic interactions between TS and the anti-cancer chemotherapeutic 5-FU (fluorouracil).

Tumour cells cannot grow in the absence of active TS, which is required for DNA synthesis. The enzyme converts deoxyuridine monophosphate into deoxythymidylate monophosphate, which is then converted into DNA's component chemicals. The body transforms 5-FU into a chemical that inactivates TS.

Solving the structure of TS has been the lead project at Agouron since the company's inception. (Source: Bio/Technology, Vol. 8, October 1990)

DNA photoaffinity label found

An oligonucleotide conjugate of 5-methyl-1,4-naphthoquinone is capable of alkylating DNA when held adjacent to a target sequence and subjected to UV irradiation, according to Moneesh Chatterjee and Steven E. Rokita of the State University of New York, Stony Brook. Although covalent reaction between DNA and free 5-methyl-1,4-naphthoquinone had not previously been detected after photolysis, it now appears that crosslinking can be induced when the quinone is forced to remain in close proximity to a target sequence of DNA. "Since this photochemical reaction essentially immortalizes the hybridization of a DNA probe", says Rokita, "reagents based on this quinone can be expected to serve as the basis for future protocols in nucleic acid manipulation and diagnosis". The conjugates could also prove useful in experimental antiviral therapy based on antisense oligonucleotides. This would involve making an antisense oligonucleotide-quinone conjugate that binds specifically and irreversibly with the RNA transcript (the "sense" strand) of a deleterious gene, thus interfering with its expression. (Reprinted with permission from Chemical and Engineering News, p. 22, 20 August 1990. Copyright (1990) by the American Chemical Society)

D. APPLICATIONS

Medical and pharmaceutical applications

Clinical trial of possible AIDS vaccine

British Bio-technology (Oxford, UK) began clinical trials during September 1990 of an immunotherapeutic agent for AIDS. The trials will be the first human test of British Bio-tech's patented virus-like particle (VLP) technology - which uses virus-like non-infectious particles from genetically engineered yeast to carry viral core or surface components. In the clinical trials, British Bio-tech will test particles carrying the p24 protein of the AIDS virus in an attempt to boost the immune response of patients. Eventually, the firm hopes to add other components of the AIDS virus to the particles, to produce a protective vaccine. The

Phase I clinical trials are being done at the Hammersmith Hospital in London with the British Medical Research Council. (Source: Chemical Week, 26 September 1990)

Babies gain from fast AIDS test

An electronic technique for detecting the AIDS virus could help diagnose the presence of the disease in babies much sooner than standard tests.

A team at the National Institute of Health in Tokyo has developed a method that is about 200 times more sensitive than ordinary checks.

Although it will probably be much more expensive, the increased sensitivity of this method should help find the AIDS antigens in infants, which is difficult using current techniques.

The method is the result of a project backed by NTT, the Japanese telecommunications giant, to find medical applications for electronic technology. Called laser magnetic immuno-assay, it mixes magnetic labels with a blood sample and uses a laser to detect how many attach themselves to the AIDS antigens.

The most commonly used tests involve chemical reactions with the virus antigens and detect levels down to 25 pg/ml. The Japanese experiment picked out a concentration of 0.1 pg/ml. (Source: Electronics Weekly, 28 November 1990)

Progress for virus vaccines?

German researchers have demonstrated that cells infected with a virus can be killed by T-lymphocytes. They recognize peptides that derive from the virus and which bind to major histocompatibility complex (MHC) class I molecules on the cell surface. The finding could lead to improved manufacture of vaccines.

According to Max Planck Institute (MPI) scientist Hans-Georg Rammensee and colleagues at MPI and collaborators at Tübingen University, "virus-infected cells produce small peptides from viral proteins, which are recognized by MHC class I-restricted CTL". Moreover, the cell produces and maintains exactly one peptide presented to a given CTL. (Source: European Chemical News, 26 November 1990)

Genentech AIDS vaccine trial

Genentech has initiated a human pilot study of its AIDS vaccine as a potential treatment for HIV infection. The product consists of a recombinant form of gp120, a protein found on the surface of HIV.

The trial aims to asess whether the vaccine can boost the immune systems of sero-positive patients. It is the first human study of the product for this indication. Earlier this year, Genentech scientists reported in Nature that inoculation with the vaccine had protected two chimpanzees from infection with HIV.

The human study will cover 55 volunteers at the Walter Reed Army Institute of Research in Washington, and will last 10 months. The trial protocol will involve comparing antibody and cellular responses in patients receiving gp120 with their own baseline and that of a control group.

Genentech is also looking at the possibility of testing the product as a potential vaccine for protecting uninfected individuals from HIV infection. (Source: European Chemical News, 10 December 1990)

"Cocktail" HIV vaccine possible

RepliGen (Cambridge, MA) has been pursuing an AIDS vaccine with pharmaceutical giant Merck (Rahway, NJ) by keying on a small identifying protein "loop" on the virus's coat. New findings that a small number of virus families exist with similar loop structures indicate that a broadly effective AIDS vaccine is feasible. Results of the study, which was performed by Duke University (Durham, NC) and Harvard (Cambridge, MA), show that a cocktail vaccine with about a dozen chemistries may be able to provide protection from most known HIV strains. (Source: Chemical Week, 12 September 1990)

Cure for sleeping sickness receives all-clear

The parasite that causes sleeping sickness, or trypanosomiasis, has a new enemy. After a long delay, the Food and Drug Administration in the US has approved a drug called eflornithine to treat the disease. Patients will start to receive the drug within months. The drug needed the FDA's approval for two reasons: first, because it originates from an American pharmaceuticals company, Marion Merrell Dow, and secondly, because the World Health Organization always seeks the Administration's approval for drugs to treat tropical diseases. The application was submitted more than two years ago.

About 25,000 people develop sleeping sickness every year. The disease is caused by trypanosomes, which are spread by the tsetse fly. If the disease is diagnosed and treated in its early stages, the patient will recover, but without treatment, the parasites can spread to the central nervous system, causing brain damage, coma and death. Once an outbreak takes hold, people evacuate the area and fertile land may lie untouched for years.

Two existing drugs for trypanosomiasis, suramin and melarsoprol, both have side-effects that can be serious and, in the case of melarsoprol, sometimes fatal. Eflornithine was originally intended for use as an anti-cancer drug. It blocks the action of an enzyme, ornithine decarboxylase, which is involved in the manufacture of amino acids called polyamides. Trypanosomes need polyamides from their hosts in order to reproduce. Trials conducted with about 600 patients suffering from advanced trypanosomiasis in Congo, Côte d'Ivoire and elsewhere have shown that the drug has no serious side-effects.

There are two drawbacks with eflornithine. First, it works only against one of the two types of trypanosome, Trypanosoma brucei gambiense, which is more common in west and central Africa. It has no effect on T. b. rhodesiense, the parasite that is found in the southern and eastern parts of Africa.

Secondly, the drug must be given intravenously, under medical supervision, so it will be licensed for use only in specialized centres, rather than in the villages where it is needed most. Nor will the treatment be cheap - a problem for the overstretched health budgets of most poor countries. The ideal

would be to transfer the technology for producing it to a developing country. (Source: New Scientist, 8 December 1990)

Harnessing biotechnology to improve the healing of wounds

Details are now available of a major UK research project designed to produce novel wound healing materials from microfungal filaments (mycelia). Not only would these offer improved clinical benefits over traditional forms of wound dressing but would also be more cost effective to produce. The two-year multi-sponsor research project is being carried out by the British Textile Technology Group (BTTG).

Previous work by BTTG has identified wound dressings as a key area of potential application for microfungal non-wovens due to the relatively high chitin and its derivative chitosan in the fungal cell walls. Furthermore, the extraction of chitin from its natural sources and its incorporation in conventional wound dressings is a comparatively costly process. Whilst the wound healing properties of chitin and chitosan, obtained from the shells of crustaceans have long been recognized, those of microfungal chitin/chitosan have yet to be established.

The microfungi Mucor mecedo and Rhizomucor miehei have been identified as having particularly high levels of chitin/chitosan in their cell walls. A suitable growth medium will be developed to maximize the biomass and chitin/chitosan yields, and the influence will be examined of carbon, nitrogen, mineral nutrients, pH and aeration conditions. Alternative organisms with an even higher chitin/chitosan content will be sought, together with a quantitative method for accurately determining chitin/chitosan content of microfungal mycelia.

The relationship between chitin/chitosan content and healing properties of the non-woven materials will be established by producing a range of microfungal materials with different proportions of chitin/chitosan and then evaluating them using tissue culture models of the wound healing environment. Production will be by manipulation of fermentation conditions and by using different species or strains of microfungi. Providing that a positive relationship is confirmed, a programme of mutagenesis and strain selection will be initiated to further increase the level of chitin/chitosan in cell walls, using specially developed rapid screening techniques.

The project will also seek to formulate mycelial materials to control the physical micro-environment at the surface of the wound. Multi-layered wound coverings will be constructed using the mycelial component to form a biodegradable wound interface layer where it can act to stimulate wound healing, prevent gross dehydration of the wound surface and facilitate dressing removal by preventing adhesion.

Composite non-wovens produced by blending microfunal mycelia with other types of fibre, believed to assist or facilitate wound healing, will also be evaluated to discover whether the component fibres can act synergistically to promote wound healing.

For further information about BTTG's research, contact Dr. Paul Hamlyn at BTTG, Shirley Towers, Didsbury, Manchester, M20 8RX. Tel: 061 445 8141. Fax: 061 434 9957. (Source: News Release, 5 November 1990)

Septic shock death rate halved

Pervasive bacterial infections can rapidly lead to life-threatening septic shock, sometimes even when conventional antibiotic treatment appears to be working. Now, according to clinical trials in the US, Canada, and Europe, an immune-based approach that uses specific antibodies to counteract septic shock caused by bacteria and their degradation products, seems to halve the death rate in serious cases. Results of tests on the product, developed by Centocor, were reported in Atlanta, Georgia, in conjunction with the annual Interscience Conference on Antimicrobial Agents and Chemotherapy.

Rapid onset septic shock is difficult to diagnose or treat, and it proves deadly about half of the time. However, antibodies that recognize the endotoxin released by gram negative bacteria as they grow or are degraded can prevent or mitigate shock in several mammalian species.

Similarly, human-type monoclonal antibodies can also recognize the bacterial endotoxin and block its action in patients, according to Craig Smith of Centocor. More than 500 patients participated in a clinical trial, and a single dose of the monoclonal antibody reduced the death rate of half, he says. The clinical results were "highly significant" and could not be "confounded by other factors".

Similar findings were obtained at clinical centres in Europe, according to Cornelis Wortel of the Academic Medical Centre in Amsterdam. Moreover, the treatment proved effective when used on a "compassionate" basis on several desperately ill children. He also notes that in clinical tests the antibody treatment reduced blood levels of factors such as tumour necrosis factor that are released during septic shock.

Applications to make the drug available for clinical use have been filed in the US and Europe, according to Smith. If approved, he says, the treatment is expected to cost $2,500 per dose. (Source: Chemistry and Industry, 19 November 1990)

British Bio-technology announces data on new anti-arthritis drugs

Results of collaborative research between British Bio-technology and SmithKline Beecham on the treatment of arthritis have been reported at the International Conference of the Inflammation Research Association.

The new compounds, known as collagenase inhibitors, help to prevent the injury to bone and cartilage in arthritic joints, a major feature of arthritis.

In animal studies reported at the meeting, the collagenase inhibitors protected bone and cartilage from inflammatory damage, reduced soft tissue swelling and decreased the overall severity of the arthritis. The two companies have been working together in arthritis research since 1987.

Chemical agents designed and synthesized by British Bio-technology have been evaluated for efficacy as anti-inflammatory agents by SmithKline Beecham research scientists in Philadelphia.

Details from: British Bio-technology Ltd., Watlington Road, Cowley, Oxford OX4 5LY. (Source: Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Antibody-enzyme conjugation kits

Cambridge Research Biochemicals have launched the IMMUNO LINK™ range of antibody-enzyme conjugation kits.

The IMMUNO LINK™ AP and IMMUNO LINK™ HRP kits provide all the reagents and gel filtration columns needed, together with a simple protocol, to conjugate two antibodies to alkaline phosphatase and horseradish peroxidase respectively.

The IMMUNO-LINK™ APL kit contains Lumi-Phos™, a stable highly sensitive chemiluminescent substrate, which provides a signal on X-ray film that will not fade with time.

The procedure takes less than two hours' hands-on time. Unlike other kits, the IMMUNO-LINK protocol contains a process control stage to ensure that quality conjugates are made every time. The covalent linkage results in conjugates stable up to six months. Conjugates of antibody fragments can also be made.

The antibody-enzyme conjugates can be used for most applications, including immunoassay, Western blotting and immunocytochemistry. Further details from: Simon Douglas, Product Manager, Bioscience, Cambridge Research Biochemicals Ltd., Gadbrook Park, Northwich, Cheshire, CW9 7RA, UK. (Source: News Release, July 1990)

AIDS vaccine is candidate for clinicals

A new candidate AIDS vaccine developed in part by Immuno AG has been granted approval for human clinical trials by the US Food and Drug Administration, the sixth such vaccine so far approved for such testing.

The vaccine was developed under a collaborative research and development agreement between the National Institute for Allergy and Infectious Diseases (NIAID), the National Cancer Institute (NCI) and Immuno-US Inc., the Rochester, Michigan-based branch of the Immuno group of companies.

The two Immuno vaccines are based on a genetically engineered, or recombinant, Rgp 160 envelope glycoprotein that surrounds the HIV-I virus. Two forms of the Rgp 160-based formula were developed and tested.

One vaccine used an adjuvant that was based on lipids, or fats, and the other used a mineral carrier-based adjuvant. Immuno says its biotechnology allows the Rgp 160 antigen to be produced on an industrial scale.

In pre-clinical studies, the Rgp 160 antigen used in the vaccine was administered to a chimpanzee, which was subsequently protected from the human immunodeficiency virus (HIV) for almost three years. The company calls this the longest known period of protection granted by any AIDS vaccine studied in animals.

The 60 human subjects to be tested will be healthy and not at risk of contracting AIDS. Researchers will be chiefly interested in learning whether the vaccine is safe, and to evaluate what kind of response it can induce from the body's immune system.

The five federal AIDS vaccine evaluation units involved are located at St. Louis University School of Medicine, the Johns Hopkins Center for Immunization Research, University of Washington School of Medicine, University of Rochester Medical Center and Vanderbilt University Medical Center.

The phase I testing will, if successful, be followed by two more phases. It may take 5 to 10 years before the vaccine has completed its testing and is ready for commercialization.

As is the case with all other approved experimental AIDS vaccines, the Immuno candidate works by tricking the body's immune system into attacking what appears to be the AIDS virus but is actually harmless.

The human subjects will not be challenged with the AIDS virus itself; rather, samples of their white blood cells will be examined in a laboratory and measured for resistance to the virus. (Source: Chemical Marketing Reporter, 3 December 1990)

Possible vaccine for Lyme disease found

Recombinantly produced surface protein from the bacterium that causes Lyme disease (Borrelia burgdorferi) is a candidate vaccine for prevention of the disease, according to Erol Fikrig and colleagues at Yale University School of Medicine. When they introduced the gene for outer surface protein A (OspA) from one strain of B. burgdorferi into Escherichia coli, the transformed bacteria produced the surface protein. Mice capable of contracting a form of Lyme disease produced antibodies to the protein after immunization with either the transformed E. coli or the recombinant protein itself. Consequently, the immunized mice remained symptom-free when challenged with any of three strains of B. burgdorferi. Protection of hamsters from Lyme disease after immunization with antiserum or inactivated B. burgdorferi had been demonstrated previously; however, the present study defines the specific protein involved. The researchers point out that although the mouse version of Lyme disease is similar to the human version, further research will be needed to determine if vaccination with OspA will be effective in humans. (Reprinted with permission from Chemical Engineering News, p. 21, 29 October 1990. Copyright (1990) American Chemical Society)

Celltech and Immunex announce production collaboration

Celltech Ltd., Slough, UK, have announced a contract with Immunex Corporation, Seattle, USA, to scale up production processes for soluble receptors. The first product will be Immunex's IL-1 Receptor (IL-1R), a genetically engineered soluble receptor fragment that binds to Interleukin 1.

Immunex is developing IL-1R to treat rheumatoid arthritis, diabetes, organ transplant rejection and

graft-versus-host disease. IL-1R binds to Interleukin 1, preventing it from binding to its natural receptor. The IL-1R produced by Celltech will be used initially in pre-clinical studies and Immunex plans to take IL-1R into clinical trials by late 1991.

Immunex Corporation was recently granted a US patent covering recombinant DNA technologies for making mammalian Interleukin 1 (IL-1) receptors and genetically engineered soluble receptor fragments (IL-1R) that bind to IL-1.

Further details from Sue Nicholls, Celltech Group plc, 216 Bath Road, Slough, Berkshire, SL1 4EN, UK. (Source: News Release, 27 November 1990)

Elephantiasis worms come out in the open

Indonesian and American scientists have scored an important victory over a parasite that disfigures millions of people around the world. Brugia malayi, one of the two parasites which causes elephantiasis, has for the first time been persuaded to grow, mate and reproduce outside a living host in laboratory cultures. The development should dramatically accelerate the pace of tests for new drugs against the disease. It should also lead to a convenient way to detect the parasite.

Two species of nematode worm, Wuchereria bancrofti and B. malayi, are known to cause the disease filariasis, the overall name for the tropical disease that can lead to elephantiasis. Mosquitoes spread the worm's larvae from person to person, where they develop into adults.

Doctors can treat the disease with a drug called diethylcarbamazine, if the infection is detected at an early stage. But an infected person must take several doses of the drug over six weeks and few people in poor countries are able to complete a course. Scientists are urgently looking for an effective drug that can be given in one dose. There are several promising compounds but tests have so far been hampered because B. malayi could only be cultured in animals.

Now a team at the US Naval Medical Research Unit in Jakarta has succeeded in culturing sexually mature B. malayi from young larvae in the laboratory. The adult worms mated and the females produced large numbers of larvae. Previously scientists have succeeded only in culturing sexually immature worms.

W.A. Riberu, Soeroto Atmosoedjono and their colleagues used a culture medium containing human serum and reagents available over the counter.

The scientists should also be able to harvest the proteins produced by the parasite, said C.P. Ramachandran who runs the World Health Organization filariasis research programme. They might then be able to develop monoclonal antibodies - pure copies of specific antibodies that could "recognize" and bind to the parasite proteins. It should also be possible to sequence the worm's genetic material and develop DNA probes. Both could lead to more convenient tests for the parasite. (Source: New Scientist, 29 September 1990)

Invention offers selective immunotherapy

An inventive bit of organic chemistry has led to devices to treat diseases by selectively altering the body's immune system. Applied Immune Sciences, Menlo Park, California, has arranged clinical testing of the devices (monoclonal antibody-coated polystyrene surfaces that selectively isolate specific human immune cells) for cancer, AIDS, and graft-versus-host disease that causes failure of bone marrow grafts. Future applications include diagnostic devices and vessels for automated syntheses of microgram amounts of polypeptides, several dozen at a time.

For cancer, the tactic is to isolate killer T-lymphocytes, activate them against the characteristic antigen, culture them to large quantities, and reinject them into the patient. In AIDS, the method isolates and amplifies the helper T-cells that produce substances that inhibit replication of the human immunodeficiency virus (HIV). And for bone marrow grafts, the technique is to remove T-cells that would attack the host before injecting the marrow.

In the past, doctors have tried to activate the immune system against certain diseases by systemic injection of interleukin-2, which activates T-cells. But as executive vice-president Thomas B. Okarma explains, systemic IL-2 exerts its effects on all immune cells everywhere in ways that are non-specific, ineffective, or toxic.

In the normal immune system, activation of cells by IL-2 occurs selectively and locally in lymph nodes. Okarma's ultimate goal is an artificial lymph node that will mimic this natural process.

The problem in inventing the new devices was to find a way to anchor monoclonal antibody molecules to interior walls of polystyrene vessels. The chemistry to do this was worked out by director of development David Okrongly. The linking molecule Okrongly finally settled upon is N-hydroxymethyl-α-bromoacetamide, made by base-catalyzed reaction of α-bromoacetamide with formaldehyde.

Clinical trials against AIDS have been in progress since December 1989 under physicians Monto Ho at the University of Pittsburgh and Ronald B. Herberman at Pittsburgh Cancer Institute. So far only anecdotal evidence of effectiveness is available. One patient experienced a return of blood cell counts to normal, reported feelings of well-being, and has maintained normal blood cell counts for six months.

Clinical trials for bone marrow transplants began in January 1990 under physician Richard O'Reilly at Memorial Sloan-Kettering Cancer Center in New York City.

Trials in cancer patients also began under physician Arie Belldegrun at the University of California, Los Angeles Medical Center. (Abstracted with permission from Chemical and Engineering News, p. 27, 22 October 1990, by Stephen Stinson. Copyright (1990) American Chemical Society)

Cetus drug fails approval

Interleukin-2, the anti-cancer drug developed by the American biotechnology company, Cetus, has failed to win approval from the US Food and Drug Administration. The decision by the FDA was a surprise as the FDA was expected to give its approval to the drug's use for treating kidney cancer. Instead, the FDA advisory panel called for further analysis of data from clinical trials. Frustration felt by Cetus at the decision was

compounded by the fact that the drug has already been approved in nine European countries. At the moment there is no approved treatment for the fatal metastatic renal cell cancer for which Interleukin-2 is aimed. (Source: BIA Bulletin, No. 5, September 1990)

Can yew stunt cancer growth?

San Antonio cancer patients will be the first in the United States to receive a new cancer-fighting drug being tested by researchers from the University of Texas Health Science Center at San Antonio (UTHSCSA). The decision to perform clinical trials in San Antonio for the agent Taxotere, developed in France from the European yew shrub, resulted from earlier UTHSCSA research on a similar drug called Taxol. In clinical trials, Taxol proved effective against large tumours, such as in ovarian, lung and breast cancer, by controlling their growth, even shrinking tumours in many cases. Taxotere, a Taxol-like compound, is in almost unlimited supply, unlike Taxol, which takes about 3,000 yews to produce one kilogram. UTHSCSA is one of the only six sites in the world approved by the National Cancer Institute to conduct initial clinical trials of prospective anti-cancer agents. (Source: Biobytes, San Antonio Biotechnology News and Information, produced by Dublin-McCarter and Associates, December 1990)

Toadstool treatment

A fungus has led to the discovery of a new group of compounds to treat tumours. Scientists at the Brigham Women's Hospital in Boston and at Harvard University found that fumagillin, an antibiotic secreted by the fungus Aspergillus fumigatus fresenius, inhibits the formation of the new blood vessels essential for the growth of solid tumours. They first saw the compound's effects when the fungus contaminated a culture of human endothelial cells.

But the antibiotic also caused severe weight loss in mice, making it unsuitable for cancer treatment. So the team synthesized and tested about 100 analogues of fumagillin, including O(chloroacetyl-carbamoyl) fumagillol, or AGM-1470, which is 50 times more active than fumagillin. AGM-1470 inhibited the growth of a variety of solid tumours in mice, including certain lung carcinomas and melanomas. The animals did not lose weight or suffer the usual side-effects of chemotherapy, such as hair loss and infections. The team says that their discovery provides a glimpse of what anti-cancer therapy might be in the future. (Source: New Scientist, 15 December 1990)

Human deathtraps for mosquitoes

If a promising line of research succeeds, some day malaria may be stamped out while inflicting poetic revenge upon one of man's oldest insect enemies. Stanford immunologist Leon Rosenberg believes it may eventually be possible to inoculate humans - not against viruses, but against mosquitoes.

Inspired by a series of studies in which cattle injected with ground-up ticks developed antibodies that interfered with the ticks' digestive processes, Rosenberg thinks he might be able to develop a similar antigen for certain mosquito species. Used in a vaccine that is harmless to people, such an antigen would quickly kill any mosquito unwitting enough to bite a vaccinated person.

The most obvious application of this technique would lie in controlling the spread of malaria. Rosenberg says. The vaccine would not protect people from the malaria parasite itself, but by inoculating malaria victims against the parasite's carrier - the anopheles mosquito - further spread of the disease could be halted.

Rosenberg is trying to find an antigen that will interfere with flea digestion so he can protect mice from their hungry parasites. If successful, he hopes to move on to the problem of malaria and mosquitoes. Rosenberg admits that even if all the scientific hurdles can be overcome, it might prove difficult to vaccinate people with something that will not actually help them: he hopes people will be amenable to "altruistic immunization". (Source: Science, Vol. 249, p. 1499, 28 September 1990 (Briefings))

Oncor develops and markets DNA probes

Oncor, of Gaithersburg, MD., has begun marketing DNA probes that can be used to identify specifically all 24 human chromosomes. The probes were developed for use with the company's in-situ chromosome analysis test system, which allows for characterizing chromosomes on an ordinary microscope slide using a fluorescence microscope. According to the company, its technique is less time consuming, costly, and labour-intensive than traditional methods. Identification of chromosomes can be used in detecting chromosome abnormalities, tracing genetic disorders, and in determining sex and sex-linked diseases. (Reprinted with permission from Chemical and Engineering News, p. 7, 22 October 1990. Copyright (1990) American Chemical Society)

Livestock applications

NIAID to test ribozyme gene therapy to combat simian AIDS

Ribozymes, one of the new approaches to gene therapy for AIDS, are being tested in "very preliminary studies" in monkey cells with Simian immundeficiency virus (SIV) as a prelude to live tests in monkeys, according to researchers at a conference on the catalytic RNA enzymes.

The co-organizers of the meeting - Nava Sarver, Chief in the Developmental Therapeutics Branch, Division of AIDS, of the National Institute of Allergy and Infectious Disease (NIAID), Bethesda, MD., and John Rossi, a research scientist in the Beckman Research Institutes, of the City of Hope, Duarte, California - reported their progress on human cell cultures with "hammerhead" ribozymes (so called because of their shape) in blocking replication of human immundeficiency virus (HIV). Sarver, Rossi and their co-workers found that, when transformed cells expressing one of the ribozymes were challenged with HIV, antigen p24 levels were reduced 50 to 100 times.

The results were impressive enough to move to monkey cells infected with SIV as a prelude to the live animal experiments.

Dusty Miller of Seattle's Fred Hutchinson Cancer Research Center suggested the best approach for gene therapy with ribozymes would be to incorporate the ribozyme-expressing genes into the bone marrow's pluripotent stem cells, which then will differentiate into all the various blood cells

that can become infected with HIV. He said in vitro experiments have shown these cells continue to churn out ribozymes as long as four months after receiving the genes.

Other methods of getting ribozymes into cells include the use of liposomes coupled with monoclonal antibodies to carry the enzymatic RNA molecules directly to the HIV-infected cells. Philip Felgner of Vical Inc., San Diego, said cationic lipids with a very high positive charge density - which his company makes - react spontaneously with anionic molecules like DNA to form a complex that is "10-fold" more effective in getting into cells than negatively charged anionic liposomes.

Sarver noted that the only successful anti-HIV strategy with documented efficacy in AIDS patients has been the disruption of viral DNA synthesis by nucleoside analogues such as AZT. However, agents that block viral function often have toxic side-effects or lose effectiveness after prolonged use. Targeting the creation of the virus in the first place by blocking the activity of viral genes at the RNA levels avoids these problems. (Source: McGraw-Hill's Biotechnology Newswatch, 19 November 1990)

Scientific support for "milk" hormone rekindles controversy

An artificially produced hormone that raises a cow's output of milk won informal scientific approval in the US. Yet opposition to its use continues to cast doubt on its future.

The hormone, which promotes growth, and is known as recombinant bovine somatotropin (rBST), has been at the centre of fervent debate for almost a decade. A copy of a natural growth hormone, rBST can boost milk production by 10 to 25 per cent when injected into dairy cows. The drugs companies Monsanto, American Cyanamid, Eli Lilly and Upjohn are trying to win approval of rBST from the US Food and Drug Administration (FDA).

The National Institutes of Health asked 13 experts to assess the data on rBST and offer an opinion on its safety in milk and its effect on bovine health. At a press conference punctuated by accusations of a cover-up, Melvin Grumbach, a paediatrician chairing the panel, said that rBST is safe.

The question of safety concerns the concentration of insulin-like growth factor 1 (IGF-1), a biochemical intermediary of growth. BST stimulates the body's production of IGF-1. Too much IGF-1 can cause enlargement of hands, feet, nose and chin, glucose intolerance and hypertension.

Cows' milk normally contains about 2 to 10 nanograms/millilitre of IGF-1 (human milk has 1 to 3 ng/ml). The rBST will raise the IGF-1 in milk an inconsequential 2 to 5 ng/ml, says Raymond Hintz, a paediatrician at Stanford University. Moreover, IGF-1 is digested in the gastrointestinal tract, and it is destroyed during preparation of milk for infant formula. Infants in the US are not fed whole milk.

The question of the effect of rBST on cows is more controversial. The panel conceded that data from the pharmaceutical companies on 20,000 treated cows are now in the hands of the FDA.

A more influential group, the Consumer Union, has also weighed in against rBST. The group, which publishes the widely read magazine Consumer Reports, says the safety of IGF-1 is still unproven. The group's own study of the data suggests that cows treated with rBST have higher disease rates, requiring more antibiotics. That could encourage resistance to antibiotics in bacteria and add to the burden of antibiotics already ingested by humans.

Milk from herds treated with rBST is now being drunk while the FDA studies its effect on cows. But Britain's Veterinary Products Committee rejected Monsanto's application to sell rBST in Britain. Norway, Sweden, Denmark, the Netherlands and parts of Canada have also banned it. (Source: New Scientist, 15 December 1990)

Agricultural applications

Cell-fusion orange developed

Kikkoman Corp., Noda, in collaboration with the Japan's Ministry of Agriculture, Forestry and Fisheries, has developed a new breed of citrus fruit by fusing cells of orange and trifoliate orange, Citrus trifoliata. Named "Oretachi", the fruit combines the winter resistance of the trifoliate orange with the flavour, texture and colour of the commercially cultivated orange. The research team is continuing with studies aimed at improving the breed further to raise its consumer appeal. Kikkoman will begin marketing seedlings next year to domestic and US citrus fruit growers. (Source: McGraw-Hill's Biotechnology Newswatch, 19 November 1990)

ESCAgenetics genetically engineers coffee

Naturally decaffeinated coffee beans may be on the menu following the news that US scientists at ESCAgenetics, based in San Carlos, California, have succeeded in inserting an antibiotic resistance gene into Coffea arabica - the species that accounts for 70 per cent of world coffee consumption.

ESCAgenetics, founded in 1976, purchased all the assets of International Plant Research Institute (IPRI), which had been operating under Chapter 11 since 1985, in 1987. The company's strategy is to develop food ingredients with lower cost or enhanced flavour, aroma or texture, and to develop cost-effective planting materials with improved yields. Coffee is a priority target. World-wide, the annual harvest of coffee is worth around £7 billion.

Details from: ESCAgenetics Corp., 830 Bransten Road, San Carlos, CA94070, USA. (Source: Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Fungus defeats ravenous rhinoceros beetle

Researchers in the Philippines have developed a fungus spore which could eradicate the rhinoceros beetle (Oryctes rhinoceros).

The rhinoceros beetle is up to 10 centimetres long with a characteristic chitinous horn. It reproduces four or five times a month, and can devastate coconut plantations in a matter of weeks. The beetle feeds on young buds and the crowns of the palm, and can reduce the yields of the tree by as much as 75 per cent.

According to Joanna Ferreira, chief technical adviser on pest control at the Philippine Coconut Authority (PCA) agricultural research and development department, the rhinoceros beetle destroys up to 35 per cent of the coconut crop each year in the Philippines. Researchers at the PCA's Davao Research Centre on the southern Philippine island of Mindanao have carried out studies of the Green Muscardine Fungus (Metharrizium anisopliae), or GMF. This fungus attacks the beetle and nothing else. When larvae are infected with the fungus they are killed within 13 days, with 68 per cent infectivity. This control lasts for about one year.

According to Ferreira: "The PCA produces the fungal spores in the laboratory in a powder form. You just mix it with coconut bait, which the beetles cannot resist, and after 30 days it will have spread through the beetle population".

Farmers can buy GMF from the PCA and apply it as a dry dust, as bait, or inject a solution into infected logs using a makeshift bamboo injector called a sumpit. They can also introduce infected larvae into breeding sites. Roughly one kilogram of fungus will cover between five and 10 hectares of coconut farm.

The Philippine Council for Agriculture, Forestry and Natural Resources Research and Development estimates that the cost of protecting one hectare, containing about 160 palms, is about 115 pesos (£2), some 260 pesos (£4.50) less than traditional control methods. (Source: New Scientist, 3 November 1990)

Approval given on fungus control

W. R. Grace (New York) has received approval from the Environmental Protection Agency to use a fungus to control a pair of common plant diseases. The Grace product - a strain of Gliocladium virens - is formulated into pellets that are applied to the soil to control two disease-causing fungi, Rhizoctonia colani and Pythium ultimum. Grace aims to commercialize its biocontrol fungus in two years. While the EPA approval is for use on greenhouse plants, Grace says it could eventually seeks federal approval for the fungus's outdoor use. Grace has exclusive license to the technology, which was developed by the US Department of Agriculture. (Source: Chemical Week, 5 December 1990)

Super-nodulation variety of soybean

A research group at Japan's National Institute of Agrobiological Resources of the Ministry of Agriculture, Forestry, and Fisheries has developed a super-nodulation variant of soybean with enhanced nitrogen-fixation abilities. The research group applied a chemical mutagen, ethyl methanesulfonate (EMS) to soybean seeds; screening of 7,000 second generation seeds yielded one plant that formed 6-10 fold more nodules than the parent strain. This is the world's third super-nodulation variant, following Australian and US successes, and is expected to be useful in developing a high-yield soybean requiring less fertilizer. The researchers are now working to improve the new variant's growth rate. (Source: Bio/Technology, Vol. 8, December 1990)

Novel champignon mushrooms developed

Hokuto Sangyo Co. Ltd. (Japan), a Nagano-based manufacturer of materials for mushroom cultivation, has developed "Hokuto#3 and #5", two new breeds of fast-developing champignon mushrooms, Lyophyllum aggregatum. Hokuto Sangyo produced the new varieties after three years of crossbreeding several hundred species of cultivated and wild champignons. They can be cultivated about three weeks faster than conventional species, and provide almost twice the quantity of harvested mushrooms. (Source: McGraw-Hill's Biotechnology Newswatch, 3 December 1990)

An end to chemical fertilizers?

A group of scientists from China and Australia claim to have found a way to make wheat seedlings manufacture their own supplies of nitrogen, with the help of a bacterium and a herbicide. If they are right, farmers will one day be able to dispense with inorganic nitrogen fertilizers.

Other scientists remain unconvinced and want to see more experiments to confirm the findings.

There is also concern because the procedure involves the herbicide 2,4-D. This could become an emotive environmental issue because of its association with Agent Orange, the defoliant that became infamous during the Viet Nam War.

Research to introduce nitrogen fixation to non-legumes, especially rice and cereal crops, has been going on around the world for many years, but with little success. In the best attempt so far, researchers in Nottingham produced nodules on the roots of wheat and rice, but none of them produced measurable amounts of nitrogen. The British researchers treated the roots of seedlings with an enzyme that weakened the walls of the root cells, allowing in the bacterium Rhizobium, which went on to trigger the formation of nodules.

Yan-Fu Nie, from Shandong University in China tried another treatment. He applied the herbicide 2,4-D to wheat seedlings. (The herbicide kills only broadleaved weeds.) The herbicide alters the development of the root in some way, again allowing bacteria to penetrate the root and multiply in the plant cells. With agricultural chemist Ivan Kennedy and microbiologist, Yau-Tseng Tchan, at the University of Sydney, Nie tried to introduce strains of Rhizobium to the roots. Like everyone else, they had no luck in achieving fixation.

Success came when the researchers abandoned Rhizobium and tried again with the bacterium Azospirillum. This time they recorded a 50-fold increase in the activitiy of the enzyme nitrogenase - a measure of how much nitrogen is being fixed. The research will be reported in Canberra at a meeting of the Australian Society for Nitrogen Fixation.

The team will work with Claudine Elmerich at the Pasteur Institute in Paris who studies the genetics of Azospirillum. Elmerich has created mutant strains of Azospirillum and will try to create a strain that is more efficient at fixing nitrogen. (Source: New Scientist, 8 December 1990)

Insect-killing cotton passes field tests

Successful first-year field trials have been carried out on cotton genetically altered to resist insect damage. The tests show that its use might allow a 35 to 40 per cent reduction in insecticide applications on cotton, and the developer, Monsanto, hopes to improve the cotton to reduce pesticide use 80 per cent.

Monsanto scientists prepared the test plants by inserting a gene from a commercially available natural bacterium, Bacillus thuringiensis kurstaki. The gene enables Bt to make a protein toxic to many caterpillar species, and the transgenic cotton makes a similar protein.

Monsanto conducted the field tests in six US states in co-operation with the US Department of Agriculture and several universities. The transgenic plants were about as bug-free as regular cotton sprayed with insecticide.

According to David Altman, a geneticist at USDA's Southern Crops Research Laboratory in College Station, Texas, Monsanto's transgenic cotton "could provide additional pest control options for cotton growers". Synthetic pyrethroids are now used to control caterpillar damage on cotton crops, notes a Monsanto spokesman, but the pests can build up tolerance to these frequently applied insecticides. Many growers spray more than 10 times a season. The genetically modified cotton would require fewer pyrethroid applications, and allow increased efficacy from the pyrethroids.

In addition, the transgenic plants are toxic only to the caterpillars that feed on the plants, and have no effect on humans or beneficial insects. Although Bt has been commercially available for the past 30 years, only frequent applications effectively controlled crop damage because it breaks down on exposure to light and readily washes off. The transgenic plants avoid these problems.

The altered plants pass the Bt gene on to succeeding generations through their seed. (Abstracted with permission from Chemical and Engineering News, p. 6, 29 October 1990, by Marc Reisch. Copyright (1990) American Chemical Society)

Food and food processing industries applications

Biocatalysts offers enhanced flavour extraction with DEPOL enzyme

Biocatalysts Ltd. has developed DEPOL NCC, an enzyme system, which can increase the yield of extracted flavours and essences from botanicals. DEPOL NCC is a blend of fungal carbohydrases that depolymerise plant polysaccharides. The action of opening up the plant tissue allows for quicker and more efficient extraction of valuable plant components. It can also be used for the breakdown of fibrous material in products such as a vegetable purees.

DEPOL NCC's B-glucosidase activity is particularly important in enhancing yield of flavour, essence or aroma as it is responsible for hydrolysing the glucosidic bond trapping the aroma components of plants within plant tissues. Releasable components of value include vanillin from vanilla pods and various monoterpenes, the aroma enhancing oils found in most fruit and vegetables. The release of monoterpenes improves the overall taste and aroma of the final product, particularly with beverages such as wines and fruit juices.

Details from: Biocatalysts Ltd., Main Avenue, Treforest Industrial Estate, Pontypridd CF37 5UT, UK. (Source: Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Cultured tastes

A California biotechnology company has developed a technology capable of producing native plant compounds in culture, and at commercially acceptable yields. Escagenetics is now providing commercial samples of culture-produced vanilla flavourings to potential partners. Similar systems for pharmaceuticals may be on the way.

Plant science pioneer Meinhard Zenk once observed that cell culture for the production of food and pharmaceuticals could have significant advantages over conventional agricultural methods. Botanical products could be produced independently of environmental factors, and to uniform specifications. Zenk also noted that the only criterion which would govern the introduction of such techniques would be "the economic aspect".

Vanilla represents the world's largest flavouring market and is worth $200 million per annum. Less than 5 per cent of products on that market are actually derived from the vanilla bean; synthetic vanillin, a secondary product from wood pulp production, accounts for the rest.

According to Angela Stafford of the UK company, Plant Science, few known plant-derived food ingredients would command a price greater than £1,000 per kg. a figure often quoted as the "break-even" point. Stafford told delegates at a London Zoological Society food biotechnology conference that natural vanilla extract, at $400 per kg, approaches this value.

Escagenetics believes the technology is directly applicable to pharmaceuticals production, and is already extending the new process to "appropriate" compounds. The company is "seeking corporate partners to develop plant cell culture production for these products".

Simmons' colleagues began working on the $6 million research project six years ago, and are now producing vanilla and vanillin in a 70-litre reactor. An undifferentiated callus culture is derived from vegetative vanilla tissue, and then transferred to a pharmaceutical grade formation reactor. The culture is stimulated with nutrients, and the product is then recovered and purified using a "food-acceptable" proprietary process. (Source: Chemistry and Industry, 3 December 1990)

"Killer cheeses" primed to fight listeria

Cheeses primed to "kill" harmful bacteria - including those that cause listeria - are now in prospect because of pioneering British research in genetics. Mike Gasson and his colleagues at the Agricultural and Food Research Council's Institute of Food Research in Norwich have identified bacterial genes that produce nisin, a natural toxin lethal to some rival bacteria.

The investigators say that their work raises the possibility of splicing the genetic material into bacteria in the "starter cultures" used for turning milk into cheese. The starter culture could then produce its own nisin, killing off other harmful strains of bacteria.

This would be a breakthrough because some soft cheeses, such as brie, may harbour Listeria monocytogenes, the species that causes listeriosis.

Infections are generally harmless with flu-like symptoms. However, they can kill people with impaired immunity and cause miscarriages.

Gasson says that his team has identified the region of genetic material within Lactococcus lactis - a relative of the bacteria used in starter cultures - which makes nisin, and has even transferred it successfully to starter cultures.

Though deadly to bacteria, nisin is not known to be harmful to people. Gasson said that nisin has been used in food with no problems for the past 15 to 20 years, mainly in processed cheese and in canning.

The team was investigating novel biotechnological approaches to enhance food safety but, as yet, had not tried making cheese with the modified cultures. The regulatory status of such cheese would need to be established before the produce could be sold. Britain is almost certain to make it mandatory in 1991 to notify the Ministry of Agriculture, Fisheries and Food of the sale of genetically engineered foodstuffs. Proposals for European regulations are also imminent. (Source: New Scientist, 8 December 1990)

Energy and environmental applications

Archaeus Technology Group's "natural" oil clean-up method

Senior oil company executives from around the world were briefed last month on a new "environmentally friendly" approach to cleaning up oil spills on beaches.

The technique has been developed by a British company, Archaeus Technology Group, and tested in collaboration with Vikoma International - one of the world's leading oil clean-up companies and one of the main suppliers of equipment to tackle the 1989 US Alaskan spill.

The approach uses a specially developed "natural" detergent or biosurfactant cultured from bacteria, which is non-toxic to plants and wildlife.

Chemical surfactants, until now the only effective method to clean up oil pollution, are banned for onshore use by the US and most European countries.

Details from: Archaeus Technology Group Ltd., Queen's Building, Kidderpore Avenue, Hampstead, London NW3 7ST, UK. (Source: Biotechnology Bulletin, Vol. 9, No. 9, October 1990)

Biological treatment for non-organic waste

A new type of biological disinfectant that can cope with mixtures of industrial, chemical and organic waste has been developed by an Italian company which claims that it has several advantages over both chemical and other biological waste treatment products.

Micro-organisms have long been used to break down organic waste, but in modern cities the sewage flow is also polluted with non-organic pollutants that bacteria cannot deal with.

However, the Italians claim that they have been able to select particular strains of natural bacteria and have improved their properties of survival in a polluted environment, enabling them not only to continue to treat organic waste but to create new ways of breaking down other chemical wastes.

The product developed by the Italians - Enzymplus - was shown at the Environtech exhibition in Hong Kong (May 1990). Its action depends on organoleptic characteristics forming the product - enzymatic compounds and several types of ciliate protozoa together with billions of living bacteria maintained in a latent state.

In this way, the waste is decomposed and metabolized by the co-ordinated action of enzymes, bacteria, protozoa, mastigophora and bacteriophages.

Enzymplus was patented in 1983 and, after several years of tests, is now being used for the treatment of animal waste (where it kills the bacteria of foot and mouth disease and allows the manure to be used for fertilizer); in municipal and industrial wastewater plants, composting and for the ecological recovery of polluted environments.

Enzymplus is available in a powder form and is applied to the surface of effluent in treatment plants. Another version is available in a water solution which provides a powerful disinfectant with strong microbiocidal action.

The makers claim that Enzymplus leaves no foul odour after its action with the wastewater; it is also usable in both aerobic and anaerobic systems. It is non-toxic and non-corrosive.

Dosage can be gradually reduced in time as there is an exponential growth of bacterial colonies and this leads to an actual reduction in the volume of sludge produced as well as reducing oxygen demand.

The disinfectant can be used over a wide range of temperatures and pH values and with low oxygen levels. It is also not inhibited by minimum quantities of carbon, nitrogen or phosphorus. (Source: ENFO, Vol. 12, No. 1, September 1990)

Industrial microbiology applications

Bacteria heat up enzymes

Researchers at Johns Hopkins University (Baltimore) have identified and characterized a pair of proteolytic enzymes from the thermophilic bacteria Pyrococcus furiosus that can survive extended periods of exposure to heat and chemicals - conditions that normally denature enzymes. The researchers say the proteases are the most thermostable enzymes yet identified, thriving in temperatures above 100°C. Because of their heat and chemical stability, the enzymes could eventually have a broad range of industrial applications, particularly if they can be made economically through genetic engineering techniques. The group signed an agreement in February with Novo Nordisk (Bagsvaerd, Denmark) on identifying and characterizing thermostable enzymes. (Source: Chemical Week, 12 September 1990)

E. PATENTS AND INTELLECTUAL PROPERTY RIGHTS

Europe changes tack on transgenic animals

The European Patent Office in Munich has rejected a decision by EPO examiners to prohibit the patenting of genetically manipulated animals. The rejection means that transgenic animals might be granted patents in Europe, as they are in the US, if

the Patent Office considers that they pose no threat to "public morality".

Harvard University applied for a European patent on any non-human mammal that has been made more likely to develop tumours by injecting an oncogene into the embryo. The "Harvard mouse" is patented in the US but the EPO ruled last year that the mouse could not be patented in Europe.

The main reason given for rejecting the application was that the European Patent Convention of 1962 prohibits patenting animals. The examiners cited the convention's exclusion in three official languages: "animal varieties", "races animaux", or "Tierarten". None of the terms is exactly equivalent.

The EPO's appeal board, however, has now ruled that this confusion did not mean that no animals could be patented, especially as the convention says explicitly that microbiological inventions may be patented. Harvard's technique, the board said, is microbiological.

But this does not mean that the appeals board supports the patenting of transgenic animals. In sending the application back to the examiners, the board also said the mice could well be excluded from patenting under another provision of the Patent Convention, one which prohibits patents on inventions considered "contrary to public morality".

The EPO examiners had previously decided that "patent law was not the right legislative tool" for resolving questions of morality. The appeal board, however, disagreed. The decision depends, they said, "on a careful weighing up of the suffering of animals and possible risks to the environment on one hand, and the invention's usefulness to mankind on the other". It said it was the job of the EPO to consider these matters when it reconsiders Harvard's case. (Source: New Scientist, 20 November 1990)

Biogen gets European α-interferon patent

After a checkered history, Biogen's patent for α-interferons has received complete approval by the European Patent Office. The patent gives the Cambridge, Massachusetts-based biotechnology company and its licensee, Schering-Plough, the right to exclude others from making and selling genetically engineered α-interferons - used in treating a number of cancers and viral conditions - in countries following the European Patent Convention.

The EPO decision was the final step in a lengthy appeal process. The α-interferon market is shared by Roche and Schering-Plough, which market their products as Roferon-A and Intron A, respectively. Intron A sales by Schering-Plough, which says it has a majority share of the world market, nearly doubled between 1988 and 1989 to about $90 million. Analysts have projected that the company's world-wide sales for α-interferon will exceed $150 million in 1990. (Abstracted with permission from Chemical and Engineering News, 26 November 1990, p. 7, by Ann Thayer. Copyright (1990) by the American Chemical Society)

CIFLA resists patent pressure

Argentina's pharmaceutical industry association CIFLA has started campaigning in both Argentina and Brazil against drug patent recognition. CIFLA is trying to lobby against pressures put on the countries' governments by the US Pharmaceutical Manufacturers Association to grant immediate intellectual property right recognition for products and processes, which were included in a list of sanctions predicted by the Super 301 Trade Act.

CIFLA is questioning whether multinational companies should be allowed a marketing monopoly in developing countries. It argues that drug prices in Argentina are lower than in the US, due to generic competition by domestic companies, benefiting from a nonrestrictive patents law. This law was scheduled to be reviewed in September 1991, when IPR will be adopted. (Source: European Chemical News, 10 December 1990)

Genetics Institute IL-3 patent

Genetics Institute (Cambridge, MA) has received a US patent covering human interleukin-3 (IL-3) type products, including proteins made using part of the IL-3 DNA sequence and exhibiting similar properties. IL-3 is a blood cell growth factor that stimulates certain cells in the body's defence system and has shown promise in treating blood cell deficiencies, including those caused by cancer treatments. Sandoz (Basel) has exclusively licensed IL-3 from Genetics Institute and initiated clinical trials with the compound in 1989. (Source: Chemical Week, 17 October 1990)

F. BIO-INFORMATICS

Biohazard testing market to become increasingly important in the United States

Biohazard testing, in the forms of human toxicity testing and environmental effects testing, has a pivotal role in industry and commerce, as the results often contribute to the initiation of standards, regulations, remediations, controls and material substitutions. Thus, labelling a chemical "toxic" has major implications and consequences.

Present biohazard tests are becoming increasingly complicated and subtle. Moreover, new types of toxicity have become recognized and added to the battery of tests that must now be considered. Clearly biohazard testing has become an emotional issue as a result of antichemical attitudes, animal rights activism and environmental protectionism.

According to a market research report written by Business Communications Company, Inc., biohazard testing will become important to a wider range of industries and product categories. Overall, the volume of biohazard testing in the United States is projected to grow at an average 2.0 per cent annual rate, increasing from about $US 679.6 million in 1990 to approximately $US 750 million by 1995. This growth will be fuelled by the testing of new chemicals, with some contributions from revisits to old chemicals previously tested and old chemicals being tested for the first time.

The share of in-house testing will decline from 76.6 per cent in 1990 to about 73.3 per cent in 1995. Outside contracting, mainly with independent testing laboratories, will claim a correspondingly increasing share, rising from about 23.4 per cent in 1990 to 26.7 per cent by 1995. This shift from in-house to outside testing is a result of major, highly visible firms taking steps to avoid publicized clashes with animal rights activists.

The report predicts that sales of products for biohazard testing in the United States will increase by about 1.2 per cent overall, from a total of $US 133.7 million in 1990 to about $US 142.1 million by 1995. Conventional animal testing, as measured in terms of animal supply, is expected to increase at a modest 1.1 per cent, from about $US 130.9 million of mammalian animal sales in 1990 to approximately $US 138.2 million by 1995.

United States sales of in vitro toxicity testing products will increase at an average annual rate of 3.8 per cent from $US 2.2 million in 1990 to about $US 3.2 million by 1995. During this period, sales of conventional products are expected to decline by an average 0.6 per cent, from about $US 1.7 million in 1990 to $US 1.65 million in 1995, resulting from an anticipated contraction in the customer base for conventional mammalian cell lines.

Newly emerging in vitro testing products will grow at an extraordinary 24.8 per cent growth rate, from a 1990 level of $US 0.5 million to $US 1.5 million by 1995. The animal rights issue, combining with the application of new technologies, are contributing to this high expected growth. At the same time, the validation process that the new in vitro products must undergo to find acceptance is slowing the pace at which they can penetrate target markets, and the lack of regulatory sanctions is thus far a significant barrier.

Envirotoxicity testing, addressing the effects of chemicals on the fauna and flora of the environment, is only minor in 1990, providing about $US 0.6 million in sales of non-mammalian animal species and plants. Envirotoxicity testing will grow at only about 2.8 per cent in the next five years, reaching about $US 0.7 million by 1995, despite concerns over chemicals in the environment.

The report "Biohazard Testing: The Business, The Issues, The Applications" is distributed in Europe by RauCon GmbH, P.O. Box 1069, W-6912 Dielheim, Germany (Phone: +49 (6222) 73562, Fax: +49 (6222) 74884) and costs $US 2,850 (plus $US 50 for air postage and handling). (Source: News Release, 8 January 1991)

ATCC announces fifth edition of the ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries

The American Type Culture Collection (ATCC) has published the fifth edition of its ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries. The catalogue lists materials deposited at the ATCC as part of the DNA repository supported by the United States National Institutes of Health (NIH), the National Institute of Child Health and Human Development (NICHD) and the National Center for Research Resources. It also lists related materials from the ATCC's own molecular biology collection and Patent Culture Depository. Details from: ATCC, 12301 Parklawn Drive, Rockville, MD 20852, USA.

Biotechnology in Japan Yearbook 1990/91

The Annual Report of Nikkei Biotechnology (Tokyo), a comprehensive assessment of the markets and research/development projects for bioindustry technologies currently being pursued in Japan, has been released for the first time in English as the **Biotechnology in Japan Yearbook 1990/91: Markets and Research/Development.**

The 930-page **Yearbook** is organized by over 150 specific product and technology categories, combined with more than 250 tables that detail the objective and stage of development for both companies and research institutes that are active in each field. Among the industries covered are pharmaceutical, chemical, diagnostic, food, agricultural and environmental remediation, with special reports, strategic analysis and historical perspective included to complete the volume.

From erythropoetin, which enjoyed sales of more than $US 140 million in its 1990 debut in Japan, to wasabi (Japanese horseradish) and tissue-cultured tofu, the details presented in **Biotechnology in Japan Yearbook 1990/91: Markets and Research Development** reveal the extent to which Japan has embraced this industry of the future.

With a domestic biotechnology market of over $US 850 million, Japan has more than 600 companies and government agencies pouring their considerable financial and human resources into the applications of biotechnology tools. Detailed, hard data on Japanese biotechnology, until now found only in the personal files of regular collaborators with Japan, is at last available with the publication of **Biotechnology in Japan Yearbook.** It is essential, timely information for all who are co-operating with or competing in Japan.

For more information, contact: Japan Pacific Associates at 467 Hamilton Avenue, Suite 2, Palo Alto, California 94301, USA.

International Biotechnology Directory 1991

The International Biotechnology Directory 1991 is the most comprehensive and up-to-date single source of information in the biotechnology industry. The Directory has been revised and expanded to feature:

- Information on almost 9,000 organizations;
- 600 new listings compared with the previous edition;
- 4,000 other updated entries;
- A comprehensive products, research and services buyers' guide.

The Directory costs £99 plus £2 for postage and packing from Globe Book Services Ltd., Stockton House, 1 Melbourne Place, London WC2B 4LF.

Current topics in marine biotechnology
(Eds) S. Miyachi, I. Karube and Y. Ishida.
Published by the Japanese Society for Marine Biotechnology, Tokyo, 1989

This volume of 437 pages contains the published proceedings of the First International Conference on Marine Biotechnology held in Tokyo from 3 to 6 September 1989. As the expression of the research activity of 400 scientists from 23 countries, it is a valuable seminal collection of papers in an emerging field of scientific research and development. A consensus definition of what is marine biotechnology cannot yet be offered, but the scope of the Conference Papers provides a ready perspective. Major sections of this Volume deal with the following areas of research and development: micro-organisms (11 papers), microalgae (24), macroalgae (17), fish, shellfish

and other marine animals (26). In addition, there are sections on supporting technology (9) and interfacial subjects (7). Two special sessions of the Conference are reported in a group of five papers concerned with atmospheric CO_2 and marine biotechnology. The two plenary lectures provide a delicate counterpoint between an American and a Japanese perspective: an eclectic set of examples of marine biotechnology at the molecular level balanced by a more theoretical analysis of science and technology devoted to the development of a life-centred culture.

A wide variety of research and development activities has been included under the general rubric of marine biotechnology. Production of foodstuffs was given emphasis, as was the production of fine chemicals. Biotechnology in this context meant more than just DNA technology. Interdisciplinarity was emphasized although much marine biotechnology occurs currently in established discipline and subject areas. Thus the papers regarding national strategies and centres for stimulating marine biotechnology are of some interest. These came from establishments in the USA, Australia, France, China and Japan.

This volume is of considerable value in providing both a recent record of what is happening in marine biotechnology and in providing access to the current diverse literature in this field. It also reveals the wide range of marine biotechnology projects under way in developing and newly industrialized countries.

Biotechnology application in wastewater treatment by P.Y. Yang and H.L. Wang. Reviewed by T. Viraraghavan

This review deals with recent applications biotechnology for the enhancement or innovation of biological wastewater treatment processes (aerobic and anaerobic). A special chapter is devoted to the use of immobilized and genetically engineered micro-organisms.

Content:

- Definition and brief history of environmental biotechnology;
- Bioprocess for wastewater treatment: fundamentals, free cell systems (aerobic systems, anaerobic systems, lagoon systems);
- Immobilized cell systems (absorbed cell systems (biofilms), entrapped cell systems, use of aquatic plants);
- Use of genetically manipulated micro-organisms in wastewater treatment;
- Overall evaluation;
- 360 bibliographical references.

This book, at $US 13 air-mailing included, is available from: Environmental Sanitation Information Center (ENSIC), Asian Institute of Technology, P.O. Box 2754, Bangkok 10501, Thailand.

Biotechnology: EEC policy on the eve of 1993

Although the EEC has the human, scientific and material resources to compete globally in the biotechnological race, it has failed so far to match strides with its main rivals - the United States and Japan. The failure may be attributed to several factors:

- Fragmentation of research efforts;
- Compartmentalization of the EEC market, as a result of disparate standards and regulations;
- Absence in the Community of the correct supportive context and infrastructure to allow biotechnology to emerge;
- Lack of a unified market, thereby discouraging companies from making the substantial investment required for the commercial and industrial exploitation of new discoveries;
- Inadequate patent protection for biotechnological inventions.

Recent developments suggest however that the Community is beginning to make the political choices needed to fulfil its potential. On 22 April 1990, the Member States adopted two Directives which go a long way towards establishing a regulatory framework for the biotechnology industry, whilst a Directive on worker protection is expected to be agreed shortly. Steps have also been taken to facilitate the development and placing on the market of high-technology medicinal products, and the "Twelve" are currently examining a proposal for a Directive on the legal protection of biotechnological inventions.

To help keep abreast of likely developments in this fast evolving sector, which is capital for the future of EEC industry, agriculture and health care, the European Study Service has just published a 400-page study entitled "Biotechnology: EEC policy on the eve of 1993". The study analyses the technical, economic and political aspects of this crucial issue and is supplemented by comprehensive annexes containing all relevant EEC documentation. The study costs 11,800 Belgian Francs, including postage and packing, and is available from European Study Service, Avenue Paola 43 - 1330 Rixensart - Belgium.

PolyCell, Inc. compiles bibliography for bispecific monoclonal antibodies

PolyCell, Inc., a subsidiary of Quest Biotechnology, Inc., announced it has compiled a scientific bibliography listing publications on the development of bispecific monoclonal antibodies and their use for research and clinical applications including diagnosis, imaging and therapy. The bibliography and copies of selected articles are available free of charge upon request by contacting Dr. Werner H. Wahl, Vice-President Science and Technology.

DNA fingerprinting manual

On 20 September, Macmillan Press published what it billed as the definitive text on one of the latest breakthroughs in molecular biology, DNA Fingerprinting: An Introduction

Written by Lorne T. Kirby of the University of British Columbia and Children's Hospital, Vancouver, the book explores the implications of this revolutionary technique - which enables scientists to identify minute tissue samples and facilitates scientific studies on the composition, reproduction and evolution of animal and plant populations.

The technique has attracted most attention as a tool for the positive identification of criminals.

Details of the book, priced at £24.95, from: Globe Book Services Ltd., Stockton House. 1 Melbourne Place, London WC2B 4LF, UK.

Cambridge Research Biochemicals' new 1991 catalogue

The new 1991 Cambridge Research Biochemicals catalogue is now available describing a wide range of "Products and Services for Life Sciences".

The 170-page catalogue is conveniently divided into colour-coded sections covering:

- Peptides, enzyme substrates and neuroactive amino acids;
- Sugars, carbohydrates and related biochemicals;
- Immunological products;
- Pin technology;
- Molecular biology; and
- Custom services (including custom synthesis of peptides, radiochemicals, antibodies, etc.).

There is also an alphabetical index, with extensive cross-referencing, to allow products to be found easily.

The catalogue contains a complete listing of all Cambridge Research Biochemicals' products and services, including many new immunological reagents and molecular biology products, and providing much more detailed information about existing products.

Copies of the catalogue are available free from Cambridge Research Biochemicals and its distributors.

Editorial contact:

Dr. D.B. Copsey
Cambridge Research Biochemicals Limited
Gardbrook Park
Northwich
Cheshire CW9 7RA, UK

Impact AgBioBusiness

CAB International and CPL Scientific announce publication of the first news quarterly to focus exclusively on the impact of technical change for the business prospects from agricultural biotechnology. Aimed at businessmen and women responsible for decision-making, Impact AgBioBusiness identifies and analyses commercial opportunities and prospects based on technical innovation in the areas of non-chemical crop protection and related biotechnologies.

Increasing concern about the use of chemicals on agriculture has led to an upsurge in research and development into alternative methods of crop protection. These include genetic engineering of plants as well as biopesticides and biological control. Numerous opportunities exist for companies and researchers to capitalize on this trend.

Over 99 per cent of crop protection is still provided using agrochemicals. However, some analysts predict that 20 per cent of all food sold will be produced without those chemicals by the turn of the century compared to less than 1 per cent today. Both the EC and the United States are putting organic food standards into law. But finding suitable alternatives to chemicals is becoming more difficult and costly.

Impact AgBioBusiness is based on the technical resources of the CAB ABSTRACTS database, a compilation of some 150,000 research summaries per year created by scientists scanning over 10,000 scientific journals, reports, conferences and books. The summaries are examined for currency and novelty and then analysed by people with particular expertise and experience in the agricultural biotechnology business sector.

The information is presented in a clear and concise form so that busy people can find the important message embedded in the vast amount of information available. Each article contains a detailed technical brief which explains and documents the basis of each analysis.

The first issue addresses bacterial insecticides, fungi to kill weeds, use of insect-killing nematodes, the prospects of biocontrol of take-all, a destructive disease of wheat, and a company review of Ecogen, a US-based biopesticide company. The next issue addresses agricultural diagnostics, genetic engineering of plants for viral resistance, the use of fungi and insects to control insects and a review of Novo Nordisk's efforts in the biopesticide industry.

Review copies are available on request from Dr. Christina Cunliffe, CAB International, Wallingford, Oxon OX10 8DE, UK.

Biotechnology directory goes on-line

A "Yellow-Pages"-style Directory of Biotechnology Information Services has just gone on-line at the National Library of Medicine (NLM), Bethesda, Maryland, USA. It contains over 1,400 separate descriptions of information resources, and will be updated monthly.

The new database lists major journals and books in the fields of biotechnology, but does not reference individual articles. It is particularly suited to scientists who want to learn what resources are available to support their research, and may also be useful commercially.

The information is assembled and maintained under contract by the bioinformatics department of the American Type Culture Collection in Rockville, MD.

The Directory is available to anyone with a modem-equipped computer and NLM user ID. The latter can be obtained free from the MEDLARS Management Section of NLM - via the Library's TOXNET (Toxicology Data Network) system. It is also obtainable as a subfile of NLM's Directory of Information Resources Online (DIRLINE).

Complex carbohydrates

The Complex Carbohydrate Structure Database (CCSD) and database management system (CarbBank) which lists carbohydrates in glycoproteins and glycolipids now has something over 3,000 records and the number of records is increasing rapidly. Plans are in hand to comprehensively cover plant and

bacterial oligo- and polysaccharides. For further information contact: Dr. Dana Smith, CarbBank Manager, Complex Carbohydrate Research Centre, The University of Georgia, 220 Riverbend Road, Athens, GA 30602, USA.

Biotechnology patent information

GENESEQ is a computer database of protein and nucleic acid sequences from published patent applications filed in the United States, Europe, Japan, and the world's other major patent offices. In addition to the sequence itself, a GENESEQ entry includes scientific and experimental information and bibliographic data specific to the patent, such as patent number and date of application. GENESEQ also contains links to Derwent Publications' other on-line patent databases, such as World Patents Index and Biotechnology Abstracts. Intelligenetics and Derwent Publications.

G. SPECIAL ARTICLE

Vaccine potency and stability: trends in technological developments

Carolyn D. Deal, Jitendra N. Verma and Bhupendra P. Doctor*

The popular saying "health is wealth" can be fully appreciated when one considers the relationship of a nation's prosperity with the health of its citizens. Diseases, especially infectious diseases, have played havoc beyond the abilities of health care management in many developing countries. However, infectious diseases are not necessarily confined to poverty stricken society. For example, Acquired Immune Deficiency Syndrome (AIDS) has crossed all social, economic and geographical boundaries. Nevertheless, developing countries are identified with diseases and sickness. Therefore the most cost effective measure of medical intervention against infectious diseases among the world's population is vaccination. In addition to being cost-effective, vaccination offers other advantages: unlike drug treatments for infectious diseases, there is no drug-resistance problem; treatment in most cases is on a one or two times basis for life; diagnoses are not always obligatory when a person is immunized, thus decreasing the financial burden; and finally, prophylaxis is always preferred to treatment.

Extensive work by the World Health Organization (WHO), the United Nations International Childrens Emergency Fund (UNICEF), and the United Nations Development Programme (UNDP) with the objective of a global childhood immunization through the Expanded Programme for Immunization (EPI) shows promising results and builds confidence in immunization as an answer to the need of a cost effective prophylaxis. Much progress has been made in the past 20 years with immunizing children against potentially fatal infections. More than two thirds of the children in developing countries receive six standard vaccines (diphtheria, measles, polio, tetanus, tuberculosis and whooping cough), preventing an estimated 2.5 million deaths each year. At the same time, an equal number of deaths are occurring each year among the remaining children in developing countries who are not immunized. Systematic vaccination has been so successful in safeguarding humans from many diseases that world-wide eradication, in the case of smallpox, and regional elimination, in the cases of yellow fever and poliomyelitis, have become realities.

For almost two centuries vaccines have been based on whole micro-organisms (either killed, inactivated or attenuated). As knowledge of the molecular organization of pathogenic micro-organisms and the function of different molecules increases, it is possible to identify protective antigens. Despite the fact that some of the early vaccines consisting of killed, whole micro-organism proved to be effective, there are several reasons why future generation vaccines should be formulated based on defined antigens: (a) to avoid hazards due to toxicity, or to genetic material that may cause replication, or in the case of retroviruses, the integration of viral genes into the infected host-cell genome; (b) to limit the number of antigens in a vaccine in order to decrease the risk of inducing auto-immune or allergic reactions; and (c) recognizing that the prospective protective antigens may break the pathogen's strategy for survival in the "hostile" immunological environment of the host.

Many of the methodological advances in the field of vaccinology and the novel approaches in the development of vaccines have occurred in the last two decades. As in any field of endeavour, developments follow advances in concepts or technology. The application of developments in the recombinant DNA technology certainly represents such advances.

In disease interventions, finding workable methods is a larger problem than acquiring available products and the technology required for production. Fortunately, industrialized nations have generously donated more than their fair share of resources towards developing vaccines that are so critical to the third world nations.

Effective use of currently available vaccines requires several additional steps, including (a) transportation, distribution, availability of proper storage facilities, and proper use of vaccines in clinics; (b) physical and biological packaging of the vaccines; and (c) thermal stability and bio-availability. Certain vaccines such as those for measles, polio, and tuberculosis contain live viruses or bacteria and must be refrigerated, thus complicating their use in developing countries. All of the commonly used vaccines must be given by injection, which requires clean needles and trained workers.

The scope of this review is to describe recent developments in vaccine technology that pertain to the factors related to improving and maintaining long-term bio-efficacy. With the advancement of

* Dr. Carolyn D. Deal is a research microbiologist at the Department of Bacterial Diseases, Division of Communicable Diseases and Immunology. Dr. Jitendra N. Verma held a National Research Council research associateship and Dr. Bhupendra P. Doctor holds the position of Director, Division of Biochemistry; all at Walter Reed Army Institute of Research, Washington, DC, 20307-5100, USA.

The opinions expressed in this article are those of the authors and do not reflect those of the Department of the Army.

recombinant DNA technology, the ability to obtain sufficient quantities of expressed-functional cloned proteins, the ability to synthesize adequately long epitopes containing peptides, the micro-encapsulation of antigens in materials such as liposomes and immunostimulating complexes, and the better understanding of how to confer more stability to antigenic material, it may now be possible to improve the bio-efficacy and stability of vaccines as well as package several vaccines into one formulation.

We have attempted to describe four different approaches that are being explored presently to package the antigenic material, to improve the bio-efficacy, to combine several vaccines into one, and to explore the possibility of improving the stability of vaccines during storage, distribution and transportation. The four approaches for packaging vaccines are (a) expression of antigenic proteins in the Vaccinia virus; (b) salmonella-recombinant vaccines; (c) encapsulating vaccines in liposomes or immunostimulating complexes; and (d) developing cloned protein and synthetic-peptide vaccines.

There are diseases for which a vaccine is not presently available. This is because a killed or attenuated vaccine could not be developed due to the lack of technology to grow the causative agents in culture on a large-scale (required for global immunizations), or because of the lack of protective immunity for attenuated organisms. The supply of antigens in large quantities may no longer be a problem. Once it is determined that a given antigen is immunogenic and generates protective antibodies, its gene can be cloned and expressed, thus producing antigen in unlimited quantities without ever going back to the original pathogen. In fact, with the use of genetic engineering, attempts are being made to obtain necessary structural modifications of these antigens to improve immunogenicity. Alternative advances in synthetic organic chemistry have provided efficient methods of synthesizing almost any peptide antigen both in the laboratory and on a large scale using industrial procedures. However, the most promising results in improving immunogenicity have been achieved by introducing the right combination of vaccine carrier and an adjuvant.

In the first part of this review we surveyed alternatives to killed or attenuated vaccines presently in various stages of development in either experimental, pre-clinical or clinical trials.

Infectious vectored virus vaccines

Most successful vaccines (e.g., smallpox, rubella, Sabin poliomyelitis, measles, mumps and yellow fever) consist of live attenuated viruses. In general, they provide long-lasting immunity, high potency, and economic manufacture and delivery. A few vaccines (e.g., Salk poliomyelitis, influenza and rabies) are killed viruses. Developments in recombinant DNA technology created new possibilities for vaccine production. Genetic engineering can be used to produce sub-unit proteins containing attenuated viruses by gene deletion or modification, or to construct live recombinant vectors. As a potential vector, the Vaccinia virus is being explored most extensively. Studies suggest that the Vaccinia virus can be used for the expression of genes from other viruses, micro-organisms and parasites and that such recombinants retain infectivity, induce humoral and cell-mediated immunity, and provide significant disease protection to experimental animals. These vectors have the ability to carry sufficient heterologous DNA to code for single or multiple genes for immunogenic proteins. (1, 2) This is because such immunizations probably present immunogenic proteins to the immune system with a conformation similar, if not identical, to the antigens presented in natural infections. In addition, vector systems permit stringent transcription and translation from inserted genes, post-translational modification and transport. (3)

Viral nucleic acids or their DNA homologues can be incorporated into host cell DNA and are known to transactivate protoncogens that could have undesirable long-term effects. Therefore, antigens lacking nucleic acids are preferred for the new generation of vaccines.

A number of groups have exploited the transfer procedure to develop the Vaccinia virus as a vector for the expression of virus or parasite antigens and inoculation with live viruses. (3, 4) Recombinant viruses are more often than not effective in conferring protective immunity. Vectored virus vaccines using the Vaccinia virus have distinct advantages: (a) they can be administered easily, often in single-dose form; (b) they are simple and inexpensive to manufacture and test; (c) they induce relatively long immunity, in most cases lifetime immunity; and (d) they are relatively stable.

Although there is no question about the efficacy of such vaccines and their safety in a great majority of recipients, they are not without problems. They have somewhat less virulent characteristics, some undesirable host ranges and complicated in vivo replication needs. (5)

Based on the knowledge gained in the last two decades and the successful insertion of one or more genes into the Vaccinia virus genome, several prominent vaccinologists proposed the insertion of multiple genes to code for the immunoreactive, protective antibody generating the antigen proteins of six (as mentioned earlier) or more paediatric vaccines. Such an "all-in-one" packaged super vaccine, if and when produced, will allow immunization of the world's population, at least children, using one-shot immunization.

Regarding the issue of stability, vectored Vaccinia virus vaccines are relatively stable. However, this can be further improved by applying techniques such as controlled gradual lyophilization.

Salmonella-recombinant vaccines

An alternative delivery system is to use attenuated bacterial strains to express foreign antigens. Recombinant DNA technology enables the identification and cloning of genes that code for specific proteins. Much of this research focuses on genes that code for important surface antigens in infectious agents. With genetic engineering, these genes may be expressed in a vector system such as Salmonella.

These systems have been advanced as potential oral delivery systems for antigens. Intracellular targeting of the antigens may be necessary to induce cell-mediated immune responses. Salmonella is a pathogen that invades the mucosal epithelium and

then spreads to cause disease. Production of an attenuated strain that will have limited invasion potential but still produce antigens is a viable possibility for a vector. Live organisms are continually producing the protective antigens and presenting them to the local immune system, thus demonstrating advantages over killed antigen vaccines.

Several approaches have been advocated for the attenuation of Salmonella. Two such approaches are mutations in the aroA locus and the galE locus. Each of these has proved to be of value and will be discussed.

Salmonella typhi strain Ty21a has been used with some success as a live oral vaccine against typhoid fever. (6-8) This strain has undergone mutation in the galE locus (UDP-galactose-4 epimerase) and is unable to synthesize a complete lipopolysaccharide in the absence of galactose in the medium. Galactose-1-phosphate accumulates and leads to cell lysis, thus making it avirulent but immunogenic. (9)

This vaccine was tested for safety and efficacy in field trials in Chile and Egypt. (6, 10) This type of vaccine is of particular interest since its route of administration mimics the natural infection. After oral ingestion, attenuated Salmonella vaccines are translocated from the intestinal lumen to an intracellular location, primarily inside macrophages, thus potentially processing antigen and stimulating a local cell-mediated immunity at the mucosal level.

One problem with Ty21a is that this strain was isolated using chemical mutagenesis, thus necessitating further work to characterize the exact nature of the genetic lesion. In addition, wide variation is seen in protection rates with this vaccine, either due to differences in virulence of various infecting strains of typhi in the field, or due to genetic changes in the vaccine strain during production. More thorough epidemiological investigation is necessary to determine the exact variables.

A second mutation of interest is in the aroA locus. Stocker et al. (11) constructed Salmonella typhi strains with non-reverting lesions in the aroA and purA genes. These mutants are dependent on aromatic compounds for growth. These strains were found to be attenuated when fed to human volunteers, causing no serious clinical reactions.

Each of these attenuated strains has been used as a background for the expression of foreign antigens from different pathogens. In 1981, Formal et al. (12) created a prototype bivalent vaccine strain by conjugal transfer of the 120-megadalton plasmid of Shigella sonnei into the galE vaccine strain Salmonella Ty21a. This bivalent strain was shown to express O antigens from S. typhi and S. sonnei. (12) This strain was shown to be safe and immunogenic in humans (13) and to stimulate an intestinal IgA response to S. sonnei in rabbits. (14) A strain of Ty21a that expressed the I form O polysaccharide was tested by Black et al. (15) Three doses of the live oral vaccine strain afforded 40 per cent protection against diarrhoea and 56 per cent protection against Hematest positive diarrhoea. However, this trial pointed out the problem in lot-to-lot variability, with only two of three prepared lots demonstrating efficacy.

Aggarwal et al. (16) demonstrated that oral immunization with an attenuated Salmonella typhimurium recombinant containing the full length Plasmodium berghei circumsporozoite gene induces protective immunity in the mouse against the sporozoite challenge. This immunity was mediated through the induction of CD8+ T cells. Similar constructs with the P. falciparum circumsporozoite gene were able to induce CD+CTLs. These results directly demonstrate the ability of an intracellular bacteria such as Salmonella to induce class I restricted CD+ CTLs and point to the potential of developing oral vaccines against malaria and other intracellular pathogens.

However, with this potential there also lie several problems. One is the stability and the level of expression of foreign antigens in the Salmonella carrier strain. This problem may be partially solved by moving the insertion site of the foreign gene from a plasmid to a chromosomal location. The second problem is the stability of the live vaccine after lyophilization and the percentage of recovery of viable cells after rehydration. The stability and recovery aspects are technical issues that must be resolved to minimize lot-to-lot variation of the efficacy of the product. Issues to be incorporated are the use of rate-controlled lyophilization and consistently effective rehydration conditions. However, the thermal stability of these products needs to be extensively explored owing to the lack of a reliable cold chain for distribution in third world countries. At present, the resolution of these variables makes Salmonella vectored vaccines an attractive approach, though currently unresolved from a practical standpoint.

Like Vaccinia virus vectors, Salmonella vectors hold monumental potential for conferring protection against a large number of diseases. However, unlike Vaccinia, the strain stability of Salmonella vectors is more of a problem. If the strain variability is resolved, it might be feasible to distribute the vaccine strain to various laboratories throughout the world for on-site production, thereby eliminating the need for a long cold chain.

Liposomes

In recent years liposomes emerged as promising vehicles for delivery of several types of vaccines. Liposomes consist of concentric vesicles of phospholipid with variable distribution of the encapsulated protein in the transmembrane interlamellar or in the lumen of liposomes, depending on the nature of the encapsulated protein. (17) Liposomes were used extensively during the last two decades as adjuvants to induce immune responses against proteins encapsulated in them. In the early stages liposomes were used to produce antibodies against protein that would otherwise be non-immunogenic. This led to the notion that liposomes induce humoral immune response. It became increasingly apparent that successful vaccines are those that are targeted to the macrophage. Liposomes are shown to be one of the competent antigen processing and presenting cells to lymphocytes. (18) Macrophages efficiently scavenge the liposomes and enzymatically carry out necessary structural alterations to make an antigen immunogenic. (19, 20)

Liposomes have many physical and chemical characteristics that might permit interesting and useful interactions with peptides and proteins; they can also be manufactured to contain lipid adjuvants such as lipid A or lipophilic derivatives

of muramyl dipeptide; and perhaps most importantly, liposomes interact vigorously with macrophages as antigen presenting cells. Some of the strategies and potential advantages of using liposomes as carriers of vaccines are summarized in table 1.

Table 1

Strategies and advantages of using liposomes as vaccine carriers

1. Possibility of conversion of non-immunogenic material to an immunogenic one.
2. Hydrophobic antigens may be reconstituted.
3. Use of relatively small amounts of immunogens.
4. Multiple antigens may be incorporated into a single liposomal preparation.
5. Adjuvants may be incorporated with antigens into the liposomes.
6. Higher titer of functional antibody activity may be achieved.
7. Longer duration of functional antibody activity may be achieved.
8. Toxicity of antigens may be reduced or eliminated by inclusion of liposomes. This applies both to toxic proteins *per se* and to allergic reactions to non-toxic proteins.
9. Soluble synthetic antigens may be presented as membrane-associated antigens in an insoluble liposomal matrix.

Fate of liposomes *in vivo*

Parenterally injected liposomes are rapidly and efficiently ingested by macrophages, while liposomes injected intramuscularly tend to remain localized for prolonged periods at the site of injection. Although the uptake of liposomes by macrophages presents an impediment in the use of liposomes as drug carriers, the uptake of liposomes by macrophages is not a detrimental phenomenon in the field of immunology. The macrophage serves as a target for delivery of liposome-encapsulated drugs and immunomodulators, and recent evidence suggests that the uptake of liposomes by macrophages is highly advantageous to the immune response against liposomal antigens. (21)

Immune response to liposomes

Antigens (proteins) encapsulated in liposomes are not yet used as vaccines to combat any disease in man or animals. Such vaccines are in developmental and testing stages. The information described here is due to the major effort by Alving *et al*. (21) and primarily concerns the antigens that consist of a synthetic peptide (Asn-Ala-Asn-Phe)4 conjugated to a bovine serum albumin or a surface protein present on the insect form of the malaria parasite *Plasmodium falciparum*. This protein was genetically cloned and expressed (R32tet32). The cloned product contains the repeating (32 times) sequence of the above-mentioned tetrapeptide. (22)

Both of these antigens were encapsulated into liposomes, either containing or lacking lipid A. Either of these antigens by itself was virtually non-immunogenic in rabbits, whereas the liposome-encapsulated antigen was highly immunogenic and the liposome containing lipid A was even more immunogenic. (17) Similar results were obtained with monkeys. (23)

Based on the striking immunogenicity of R32tet32 in liposomes containing lipid A in rabbits and monkeys, its efficacy for production of antibody was tested in humans. (23) Preliminary results confirm the absence of significant acute toxicity of the vaccine and the occurrence of a very strong humoral immune response. A non-toxic liposome-lipid A formulation having potent adjuvant properties for inducing immunity to a liposomal antigen may therefore be achieved in humans.

Immunostimulating complexes and matrix

Immunostimulating complexes (ISCOMs) are morphologically identified as symmetrical cage-like structures of some 40 nm in diameter with hexagonal or pentagonal subunits of 12 nm. The sedimentation coefficient is 19 S as compared to 30 S for the glycoprotein micelles, which are sub-microscopic particles with a built-in adjuvant. The antigen is attached to a ISCOM matrix via hydrophobic interaction. At least one hydrophobic domain must therefore be present within the antigen. (24) The unique component of ISCOM matrix is Quil A, a triterpenoid with two carbohydrate chains. The other specific and essential component is cholesterol. (25) To encapsulate a protein antigen, a lipid less rigid than cholesterol (e.g., phosphatidyl choline) may be included in the ISCOM matrix. The final formulation of an ISCOM with an optimal amount of protein consists of Quil A, cholesterol, phosphatidyl choline and protein in an equimolar ratio. Generally, envelope proteins of viruses can be included according to the above formulation. Other hydrophobic molecules, like outer membrane proteins (OMP) of bacteria, are more difficult to handle and may require special solubilization systems. These formulations are stable and their stability may be explained by an energetically favourable symmetrical assembly of the particles.

The immunogenicity of ISCOMS

Generally, the antibody titers induced by ISCOMS have been tenfold higher, or even more, than the titers induced by the same antigen in either a killed micro-organism or a micelle form. The same antigen in a monomeric or undefined form induces even lower antibody titers. (26, 27) Oligopeptides or other small molecules in general show properties like haptens and are therefore dependent on a carrier molecule. Such a carrier, in this case hemagglutinin of the influenza virus, can be integrated into ISCOMS. (28) Such an ISCOM-carrier molecule makes the integrated small molecule strongly immunogenic. Immunostimulating complexes (ISCOMs) have been prepared from influenza A virus envelope glycoproteins; (29) i.e. hemagglutinin (HA) and neuraminidase (NA).

Chemically synthesized vaccines

The development by Merifield (30) of methods to chemically synthesize pieces of proteins (peptides) has opened up the possibility of developing totally synthetic vaccines. Vaccines such as these offer the potential of eliciting protective antibodies to well defined components while minimizing harmful

side-effects due to other components contained in the whole vaccine. However, important issues such as the most effective presentation of peptides to the immune system and the stability of these preparations must be addressed before the full promise of these vaccines can be realized.

Early work in this area centered around virus systems. The concept of immunizing with a portion of a protein to elicit neutralizing antibody was demonstrated by Anderer (1963) in the tobacco mosaic virus system. (31) This was extended by Anderer and Schlumberger, (32) who showed that a synthetic peptide from this region would also elicit neutralizing antibodies. Similar observations were made in other viral systems such as MS2 bacteriophage (33) and foot and mouth disease virus. (34) Taken together, these reports heralded the opening of the field of synthetic peptide vaccine technology.

One example of this approach is that of the foot and mouth disease virus. A variety of experiments point out the potential as well as the problems inherent in synthetic vaccines. One of tne first problems is identifying potential immunogenic sites on the protein. The approach, taken by Bittle et al., (35) was to synthesize peptides representing the entire sequence of the VP1 protein from the foot and mouth disease virus. The amino acids 141-160 region produced high levels of neutralizing antibody that protected the animals against challenge. However, this region of the protein is highly variant from serotype to serotype and points out the problem of antigenic variation, which is inherent in many of these systems.

Clearly one of the major problems encountered in synthetic peptide vaccine development is the method of delivery. (36) Initial experiments have been carried out with peptides chemically coupled to a carrier protein. However, the choice of carrier is problematic because of the repeated immunizations needed by a patient with these carriers. Tetanus and diphtheria toxoids have been suggested as appropriate carriers, but the problems of lot-to-lot variation in coupling and the exact analysis of the peptide's coupling site make this a less desirable choice.

Later studies focused on immunization with polymerized peptides to increase the size of the molecule to that of a protein without the use of a carrier protein. However, the selection of peptides as good B-cell antigens must also be coupled with the addition of T-cell antigens. An alternative to polymerization is the idea of multiple copies of an epitope presented on a branched chain core. (37) This also eliminates the need for a carrier protein.

Another presentation or delivery system is to incorporate the peptides into liposomes. The liposomes could enhance the proper presentation of the peptide so as to increase its immunogenicity and also improve the reactivity to the native protein or disease producing organism. Experiments by Alving et al. (38, 39) on malaria sporozoite antigens suggest that antibodies made to peptides presented in this fashion react with the intact parasite. In addition, liposomes introduce the possibility of incorporating adjuvants such as Lipid A into the formulation owing to the reduction in pyrogenicity seen once the Lipid A is in the liposome.

All these methods have looked at injection as the route of administration. Oral immunization is of great practical concern, both from the point of view of the ease of administration and the possibility of inducing a secretory response that may be of particular importance in diseases of mucosal surfaces. Here, protection of the antigen during passage through the stomach is important. Liposomes as well as micro-encapsulation, two slow dissolving polymers, offer a good potential.

In all of the examples discussed, the ultimate problem is stability and delivery of an intact product. This is not an easy problem, and it is one in which technology must advance. Delivery of vaccines to third world countries must not have to depend on a reliable cold chain. Lyophilization of products for subsequent rehydration depends on a reliable, safe water supply. Many products do not reconstitute well following conventional lyophilization. Particularly for synthetic peptides, the maintenance of epitope integrity after rehydration is a key issue. Growing technology in this area includes the application of rate freezing and controlled lyophilization. Studies need to be done to define the parameters of preparation, storage and shipment, and their effects on product efficacy.

In other respects, eliminating the need for rehydration by developing products that can be safely shipped at ambient temperatures and taken orally offers the ultimate in ease and reliability. Materials such as cyclodextrins that serve as a pocket for the peptide to fit into are a potential alternative. They provide the advantages of stability under environmental extremes and also increase the solubility of the peptides. Clear advances in these areas are needed before the products of modern technology can be broadly distributed throughout the world.

In conclusion, the advances in modern technology hold out mankind's dream to prevent infectious diseases, yet practical concerns need to be resolved before the dream is realized. Cloned products are continually generated and are tested as vaccines. Some are found to be useful, however much efforts need to be expended. Of particular importance is testing for bio-efficacy and thermal stability of potential vaccines under a variety of environmental conditions. There are problems encountered with several of the candidate antigens. These include, solubility of cloned product, lack of proper combination with the adjuvant, thermal stability and stability during transportation. Variation in bio-efficacy of the cloned product as a vaccine or even as a biologically active protein is observed when stored in concentrated frozen form, lyophilized form, or stored at various temperatures (-70°, -20°, 0°C and at room temperature). Detailed studies of each of these storage conditions need to be undertaken so as to standardize the bio-efficacy of the final product. Vaccines such as the recombinant product or others that transfer genes to the Vaccinia virus or to Salmonella promise to be the most broadly useful in the future. Further work is both merited and mandated.

A second area where research effort should be directed are the future generation packaging materials, such as liposomes, ISCOMS, cyclodextrins and microcapsules. They hold the promise of providing thermal stability to products. In addition, they may allow the antigen to mimic solution conformation by providing the proper combination of hydrophobic and hydrophilic environments. This imitation of nature may be the key to success with these materials.

REFERENCES

1. Perkus, M.E.; Piccini, A.; Lipinskas, B.R.; Paoletti, E. Recombinant Vaccinia Virus: Immunization against multiple pathogens. (1985) Science, Vol. 229, pp. 981-984.

2. Smith, G.L.; Moss B. Infectious poxvirus vectors have capacity for at least 25,000 base pairs of foreign DNA. (1983) Gene, Vol. 25, pp. 21-28.

3. Moss, B. Vaccinia virus expression vectors. **Progress in Vaccinology** (1989) G.P. Talwar, Ed., Springer Verlag, New York, pp. 415-422.

4. Kieny, M.P.; Lathe, R. Vaccinia recombinants expressing foreign antigens: Antiviral and antitumour immunity. **Progress in Vaccinology** (1989). G.P. Talwar, Ed., Springer Verlag, New York, pp. 423-428.

5. Dowd, P.S.; Heatley, R.V. The influence of undernutrition on immunity. (1984) Clincal Science, Vol. 66 No. 3, pp. 241-248.

6. Wahdau, M.H.; Serie, C.; Cerisier, Y.; Sallam, S.; Germanier, R. A controlled field trial of live Salmonella typhi strain Ty21a oral vaccine against typhoid: Three year results. (1982) Journal of Infectious Diseases, Vol. 145, pp. 292-295.

7. Edelman, R.; Levine, M.M. Summary of international workshop on typhoid fever. (1986) Review of Infectious Diseases, Vol. 8, pp. 329-349.

8. Hirschel, B; Wiithrich, R.; Somaini, B.; Steffen R. Inefficacy of the commercial live oral Ty21a vaccine in the prevention of typhoid fever. (1985) European Journal of Clinical Microbiology, No. 4, pp. 295-298.

9. Germanier, R. Isolation and characterization of GalE mutant TY21a of Salmonella typhi: A candidate strain for a live, oral typhoid vaccine. (1975) Journal of Infectious Diseases, Vol. 131, pp. 553-558

10. Levine, M.M.· Herrington, D.; Murphy, J.R.; Morris, J.G.; Losensky, G.; Tall, B.; Lindberg, A.A.; Svenson, S.; Bagar, S. and Edwards, M.F. Safety, infectivity, immunogenicity, and in vivo stability of two attenuated auxotrophic mutant strains of Salmonella typhi, 541 ty and 542 Ty as live oral vaccines in humans. (1987) Journal of Clinical Investigation, Vol. 79, pp. 888-902.

11. Stocker, B.A.D.; Hoiseth, S.K. and Smith, B.P. Aromatic dependent Salmonella species as live vaccines in mice and calves. (1983) Development of Biological Standardization, Vol. 53, pp. 47-54.

12. Formal, S.B.; Baron, L.S.; Kopecko, D.J.; Washington, O.; Powell, C.; Life, C.A. Construction of a potential bivalent vaccine strain: Introduction of Shigella sonnei form I antigen genes into the GalE Salmonella typhi Ty21a typhoid vaccine strain. (1981) Infection and Immunity, Vol. 34, pp. 746-750.

13. Tramont, E.C.; Chung, R.; Berman, S.; Keren, D.; Kapfer, C.; Formal, S.B. Safety and antigenicity of typhoid-Shigella sonnei vaccine (strain 5076-1C). (1984) Journal of Infectious Diseases, Vol. 149, pp. 133-136.

14. Keren, D.F.; Collins, H.H.; Baron, L.S.; Kopecko, D.J.; Formal, S.B. Intestinal immunoglobulin A responses in rabbits to a Salmonella typhi strain harbouring a Shigella sonnei plasmid. (1982) Infection and Immunity, Vol. 37, pp. 387-389.

15. Black, R.E.; Levine, M.M.; Clements, M.L.; Losonsky, G.; Herrington, D.; Berman, S.; Formal, S.B. Prevention of shigellosis by a Salmonella typhi-Shigella sonnei bivalent vaccine. (1987) Journal of Infectious Diseases, Vol. 155, pp. 1260-1265.

16. Aggarwal, A.; Kumar, S.; Jaffe, R.; Hone, D.; Gross, M.; Sadoff, J. Oral Salmonella: Malaria circumsporozoite recombinants induce specific CD8+ cytotoxic T-cells. (1990) Journal of Experimental Medicine, Vol. 4, pp. 1083-1090.

17. Alving, C.R. Liposomes as carriers of vaccines: **Progress in Vaccinology.** G.P. Talwar, Ed., Springer-Verlag, New York, pp. 429-438.

18. Alving, C.R. Delivery of liposome-encapsulated drugs to macrophages. (1983) Pharmacological Therapeutics, Vol. 22, pp. 407-424.

19. Roerdink, F.; Wassef, N.M.; Richardson, E.C.; Alving, C.R. Phagocytosis of liposomes opsonized by complement: Effects of negatively charged lipids. (1983) Biochimica Biophysica Acta, Vol. 734 pp. 33-30.

20. Wassef, N.M.; Alving C.R. Complement-dependent phagocytosis of liposomes by macrophages. (1987) Methods in Enzymology, Vol. 149, pp. 124-13

21. Alving, C.R. Liposomes as carriers for vaccines, in Ostro M. (ed.): (1987) **Liposomes: From biophysics to therapeutics.** New York, Marcel Dekker, pp. 195-218.

22. Ballou, W.R.; Hoffman, S.L.; Sherwood, J.A.; Hollingdale, M.R.; Neva, F.A.; Hockmeyer, W.T.; Gordon, D.M.; Schnieder, I.; Wirtz, R.A.; Young, J.F.; Wasserman, G.F.; Reeve, P.; Diggs, C.L. and Chulay, J.D. Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. (1987) Lancet, Vol. 1, pp. 277-281.

23. Alving, C.R.; Richards, R.L. Liposomes containing lipid A: A potent non-toxic adjuvant for a human malaria sporozoite vaccine. (1990) Immunology Letters, Vol. 25, pp. 275-280.

24. Morein, B.; Sundquist, B.; Hoglund S.; Dalsgaard, K. and Osterhaus, A. ISCOM, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. (1984) Nature, Vol. 308, pp. 457-460.

25. Lovgren, K. and Morein, B. The requirement of lipids for the formation of immuno-stimulating complexes (ISCOMS). (1988) Biotechnology and Applied Biochemistry, Vol. 10, pp. 161-172.

26. Morein, B.; Helenius, A.; Simons, K.; Pettersson, R.; Kaariainen, L. and Schirrmacher, V. Effective sub-unit vaccines against enveloped animal virus. (1978) Nature, Vol. 276, pp. 715-718.

27. Morein, B. and Simons, K. Sub-unit vaccines against enveloped viruses: virosomes, micelles and other protein complexes. (1985) Vaccine, Vol. 3, pp. 83-93.

28. Lovgren, K.; Lindmark, J.; Pipkorn, R. and Morein, B. Antigenic presentation of small molecules and peptides conjugated to a preformed ISCOM as carrier. (1987) Journal of Immunological Methods, Vol. 98, pp. 137-143.

29. Morein, B.; Lovgren, K.; Hoglund, S. Immunostimulating complex (ISCOM) in: **Immunological adjuvants and vaccines.** (1989) G. Gregoriadis, A.C. Allison, J. Poste, Eds.; Plenum Press, New York, pp. 153-162.

30. Merrifield, R.B. Solid phase peptide synthesis I. The synthesis of a tetrapeptide. (1963) Journal of the American Chemical Society, Vol. 85, pp. 2149-2154.

31. Anderer, F.A. Versuche zur Bestimmung Der serologisch terminaten Gruppen des Tabakmosaikvirus. (1963) Zeitschrift Nature, Band 188, Seite 1010.

32. Anderer, F.A. and Schlumberger, H.D. Properties of different artificial antigens immunologically related to tobacco mosaic virus. Biochimica Biophysica Acta. Vol. 97, pp. 503-509.

33. Langebeheim, H.; Arnon, R.; Sela, M. Antiviral effect on MS-2 coliphage obtained with a synthetic antigen. (1976) Proceedings of the National Academy of Sciences, USA. Vol. 73, pp. 4636-4640.

34. Bittle, J.L.; Houghten, R.A.; Alexander, H.; Shinnick, T.H.; Sutcliffe, J.G.; Lerner, R.A.; Rowlands, D.J. and Brown F. Protection against mouth disease by immunization with a chemically synthesised peptide predicted from the viral nucleotide sequence. (1982) Nature, Vol. 298, pp. 30-33.

35. Bittle, J.L.; Worrell, P.; Houghten, R.A.; Rowlands, D.J. and Brown, R. In **Vaccines 84: Modern Approaches to Vaccines** (1984) R.M. Chanock and R.A. Lerner, eds., pp. 103-107, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

36. Brown, F. The development of chemically synthesized vaccines. (1989) Advances in Veterinary Science and Comparative Medicine, Vol. 33, p. 173-193.

37. Tam, J.P. Synthetic peptide vaccine design: Synthesis and properties of a high density multiple antigenic peptide system. (1988) Proceedings of the National Academy of Sciences, USA, Vol. 85, pp. 5409-5413.

38. Richards, R.L.; Hayre, M.D.; Hockmeyer, W.T.; Alving, C.R. Liposomes, lipid A, and aluminium hydroxide enhance the immune response to a synthetic malaria sporozoite antigen. (1988) Infection and Immunity, Vol. 56, pp. 682-686.

39. Richards, R.L.; Swartz, G.M.; Schult, C.; Hayre, M.D.; Ward, G.S.; Ballou, R.; Chulay, J.D.; Hockmeyer, W.T.; Berman, S.L.; Alving, C.R. Immunogenicity of liposomal malaria sporozoite antigen in monkeys: Adjuvant effects of aluminium hydroxide and non-pyrogenic liposomal lipid A. (1989) Vaccine, Vol. 7, pp. 506-512.