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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

# Genetic Engineering and Biotechnology Monitor

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Special in this issue: An article on Bacterial Leaching: A potential for Developing Countries by Rohini Acharya, International Federation of Institutes for Advanced Studies, Maastricht, the Netherlands.

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## A. POLICY, NEWS AND OTHER EVENTS

### UNIDO news

#### Microbiology courses at International Centre for Genetic Engineering and Biotechnology

Recombinant DNA and computers for molecular biology were on the syllabus of this summer's efforts to bring the latest techniques in genetic research to the third world. Trainee scientists attended the first two courses being offered by the International Centre for Genetic Engineering and Biotechnology (ICGEB) at its laboratories in New Delhi and Trieste.

Molecular biology of chloroplasts - dealing with recombinant DNA technology for plant cells containing chlorophyll - was the focus of the course running in New Delhi (3 July - 10 August), attended by trainees from as far afield as Cuba, Hungary, Iraq, Mexico and Pakistan. Lead by the component's Head, K. K. Tewari, it drew on the teaching expertise of international scientists who are undertaking research at the Centre.

The aim is to prepare participants to isolate DNA, construct a recombinant DNA library, restriction map and sequence genes, identify proteins by antibody binding, light activate genes and the like. The course has been organized so that trainees will be able to use the latest techniques in their own countries while teaching their colleagues.

As a follow-up, ICGEB will maintain contacts with these scientists as well as provide them with advice. Three such courses on different topics are envisaged for New Delhi annually.

In Trieste, meanwhile, 41 students from 14 ICGEB member countries have recently taken part in a course on computer applications in molecular biology (3-13 July) to show them how electronic automation could allow them quicker access to the increasing wealth of data on DNA.

Combining both teaching and research, the course was designed to encourage trainees to apply software to their own research material, such as DNA and protein sequences, which they brought to Trieste.

Organized by D. Brutlag of Stanford University, it was conducted by leading scientists in computer science and molecular biology, including J. Collins of Edinburgh University, A. Bairoch of Geneva University and C. Sander of EMBL Heidelberg.

Due to the favourable response by participants, the course will be repeated next year. They also called on the Centre to organize a central laboratory for services to member countries in biological sequence retrieval and analysis. (Source: UNIDO News Release, 24 July 1989)

### United Nations and other organizations' news

#### Biotechnology aid

The UN Food and Agriculture Organization has committed itself to supporting more research into biotechnology both in and for the third world. During a symposium entitled "Plant Biotechnologies for the Developing World" in Luxembourg organized by the FAO, representatives listened to scientists from the richest and poorest countries in the world debating whether biotechnology is an "appropriate" science that should be carried out in the third world. (Source: New Scientist, 8 July 1989)

#### International pesticides register proposed

Some one million people a year suffer acute poisoning from pesticides, and 20,000 people a year die from pesticide poisoning, according to a report by the World Health Organization in 1986. Pesticide poisonings are increasing as developed countries sell lethal chemicals to developing countries that have no adequate safeguards to protect their people, livestock or wildlife. The Food and Agriculture Organization says very toxic pesticides are available in at least 85 developing countries. Some 80 of those countries have no adequate system to approve or monitor the toxins. World pesticide sales have nearly doubled since the mid-1970s to $18 billion a year currently. Much of the growth has occurred in the third world. Doctors and health workers have reported many cases of workers who wear no protective clothing because of the heat, use of empty pesticide containers to store water and food, and use of pesticides as shampoos to kill head lice.

A UN committee has now proposed an international pesticide register. Countries would indicate what pesticides would be accepted and which would be refused entry into their country. The US exports 500 million pounds a year of pesticides that are banned or restricted in the US. Exporters are required to notify the US Environmental Protection Agency (EPA) about such exports so that it can notify the receiving country, but the Agency says that only 10 per cent of exports are actually reported. (Extracted from New York Times, 30 May 1989)

### Social Issues

#### Nordic programme for biotechnology and Swedish review

Within the framework of Nordic co-operation involving the Scandinavian countries and Denmark, a joint biotechnological programme has been initiated. The Nordic Council of Ministers has instituted a special commission for ethics in biotechnology.

Research on genetic engineering, its development and practical application to plants and animals, will be the subject of a review in Sweden, according to an announcement made by the Ministry of Agriculture. The study will include an account of the potential and risks.

Under current legislation, the Swedish Government can prohibit or stimulate conditions for the use of genetic engineering on animals. A proposal for similar rules for plants is now being worked out at the Ministry. (Source: BIO Technica Journal, No. 2)

### General

#### OECD report says biotechnology's major impact yet a decade away

Biotechnology could, through rapid scientific and technical advances, become a net creator of employment beyond the next decade. Among developments contributing to this trend will be an increase in the number of new (rather than substitution) products in industry, innovations in environmental protection, and the emergence of novel agricultural crops. The number of jobs in R&D will increase over the next 10 years, too, although cost reduction policies will mean that overall employment in biotechnology companies is unlikely to grow. Beyond the turn of the century, biotechnology could begin to play an economic and social role comparable

to that of information technology and then, in the second decade of the next century, have major macro-economic impacts.

These are among the conclusions of the Office of Economic Co-operation and Development's (OECD) latest biotechnology report, "Economic and Wider Impacts of Biotechnology". The study completes the Committee for Scientific and Technological Policy's work, initiated at the time of the first OECD report in 1982, on long-term impacts of biotechnology.

The new report also draws on the results of a 17-country survey of 94 companies with interests in biotechnology.

Reviewing the "potentially very broad range of applications for new biotechnology", the report warns that "the actual range is much narrower" and points out that during the last two to three years, companies have become more concerned with technical limits, costs and market demand.

In addition to better international harmonization of patent protection (which is "essential for the large scale diffusion of biotechnology"), the report identifies public confidence as the potentially principal factor determining its rate of acceptance. (Extracted from Bio/Technology, Vol. 7, May 1989)

The growing field of biopesticides

Increasing unpopularity of chemical pesticides is opening up opportunities for their biological cousins, and the potential for improving the bioproducts through genetic engineering has opened a large, promising market that a range of players - from start-up biotechnology companies to the major pesticide producers - are rushing to attack.

The field comprises insecticides based on bacteria and viruses, herbicides based on fungi, and fungicides based on bacteria. Given that anxieties about chemical crop-protection agents centre on insecticides and that bio insecticides based on Bacillus thuringiensis (Bt) have been marketed worldwide since the 1970s, it is no surprise that that is where the most progress is currently being made.

Efforts to improve biopesticides' efficiency and minimize disadvantages should raise the Bt market tenfold during the next decade from the current $40 million a year level, 0.4 per cent of the world pesticide market. Bt's high selectivity - for caterpillars, beetles, or mosquitoes, depending on the strain - was once viewed as limiting but is increasingly being seen as an advantage. Mycogen president Jerry Caulder thinks an eventual 10 per cent market share for biopesticides is realistic.

The US Environmental Protection Agency (EPA) views the biopesticide trend as "environmentally sound", and hopes to begin registering biological products that meet its testing requirements soon.

Bt products do not yet show signs of encountering insect resistance and the regulatory requirements are indeed proving less onerous.

Biotechnology techniques also open the way to improving Bt strains by cross breeding by widening the range of insects they can control.

The major international pesticide companies are reckoned by observers to be without exception involved in biopesticide work, though most of that is shrouded in secrecy. "Conferences on biopesticides are a dead loss", says a source at a leading chemical company. "Everyone comes to listen and nobody to speak." (Extracted from Chemical Week, 28 June 1989)

Biotechnology business

Over the past decade hundreds of tiny biotechnology firms have sprung up and new ones are still being created almost every week. Few have ever made a big, profitable product. Several hundred new products are in the pipeline, but the industry's bosses admit these are unlikely to make money for years. The cumulative turnover for some 400 American start ups amounts to just $1 billion. Only one firm - Genentech - makes a sustained profit and even that is disappointingly small. Yet investors continue to pour money into these companies on a heroic scale - $10 billion so far. How much longer can this industry defy gravity? The amazing answer is: quite a bit longer.

Without a doubt, biotechnology is one of the key technologies of the future. It permits the transfer of genes, the factors which control the synthesis of all proteins, from any living organism to another. Biotechnologists can also tinker with genes themselves. For investors, the greatest attraction is the industry's potential for inventing lots of wonderful new drugs, each of which may generate yearly revenues of $500 million.

After a decade of research lots of new drugs have been discovered. On the way are growth factors, which can help heal gaping wounds, or effective treatments for ailments such as arthritis and heart disease. Drugs invented for one disease often prove unexpectedly useful for something else.

Ever since the industry's birth, biotechnologists have used such promises to lure cash. The first to take the bait were venture-capitalists, fresh from triumphs in computers. But biotechnology proved different: it takes ages to get products to market and the early arrivals were not as had been hoped. These funds still made money because fresh investors arrived; even without products biotechnology firms could still be brought to the stockmarket.

Despite the almost total lack of profits among biotechnology start-ups, about 150 have gone public (a number have even pulled off secondary share offerings), raising an additional $4 billion beyond their initial venture capital funding. Venture capitalists still hand over sums as large as $5 million to individual academics. Other sources range from R&D limited partnerships, where rich private investors in America give money to exploit tax breaks, to private placements, to finance from much bigger companies eager to obtain access to biotechnology. According to Shearson Lehman Hutton, a New York firm of stockbrokers, by February 1989 more than 28 big firms owned shares in 26 of the 46 biotechnology start ups it surveyed.

In the end, biotechnology will make money. But what most investors in biotechnology start-ups may have overlooked is that, even if it survives, the small firm which invents a new drug will not collect most of the cash its innovation eventually

generates. The ones that will collect the big profits will be the already-giant drug companies, with their huge development budgets and marketing muscle.

Most biotechnology firms have underestimated the expense and time it will take to bring products to market. At first they predicted that their products would rush through drug-approval systems because they were similar to naturally occurring substances. Now it is clear that the US Food and Drug Administration (FDA) has decided to treat biotechnology products as conventional drugs. In some cases, such as growth factors, the FDA is demanding even more stringent testing.

More important, during the past decade the profitable lifetime of drugs has declined while the costs of testing and marketing, which must now be worldwide in order to recoup big investments, have escalated. Few biotechnology start-ups are, or ever will be, able to afford to develop, manufacture and launch their new products. Even the big drug companies think they may not be big enough - witness the recently announced merger between SmithKline Beckman and Beecham, which the companies said was aimed at economies of scale.

In the next ten years, this is how the biotechnology industry will look:

- Most of the profits will be made by established, large firms that can afford to take biotechnology products to market.

- Only a handful of biotechnology firms will generate annual revenues of $1 billion-plus. They include those like Genentech or Amgen which have already built up a large sales force.

- Remaining companies will turn into research boutiques, working on behalf of the traditional pharmaceutical industry and bigger biotechnology firms. A growing proportion of drugs are invented by these small, academic outfits. Unlike development, research seems to work better on a smaller scale. On average, it costs some $30 million to invent a new drug, but $70 million to conduct the tests that are needed to develop it - never mind marketing costs. The average biotechnology firm has formal links of one kind or another with six other companies. Not only has this web of tie-ups spread costs and risks, but it has also helped protect many biotechnology firms from takeovers.

- Others will concentrate on specialized markets. Venture capitalists will now back only start-ups with identifiable goals: human-gene therapy (turning genes into drugs), drug design and so on.

- Many biotechnology firms will go under - particularly once the courts clarify chronic confusion about what constitutes a biotechnology patent. Because many biotechnology products resemble naturally occurring proteins and genes, patent officers have found it difficult to decide what is patentable. But once they sort that problem out, the high hopes of scores of smaller companies currently working on similar products will go up in smoke. (Extracted from The Economist, 13 May 1989)

Monsanto funds biotechnology effort

More than 2,000 St. Louis area students will soon be learning about genetic engineering, gene splicing and other aspects of biotechnology as the result of a grant announced last week by Monsanto Fund.

The grant will be used to expand a one-year-old biotechnology curriculum programme which is one of the first in the country geared to students below the college level. The programme - which Monsanto says could become a national model - is being offered to students from the sixth grade to the high school level in St. Louis and St. Louis county.

The grant for $200,000 follows a $40,000 grant from Monsanto Fund in 1987 to fund the development of the curriculum and its initial use in a pilot programme. The recipient of both grants is the mathematics and science education centre, a non-profit organization which seeks to improve the level of mathematics and science education in private and public schools in the area. (Source: Chemical Marketing Reporter, 1 May 1989)

International racket involving rare orchids

Orchids made their first appearance at the Old Bailey. Some of the world's most endangered species were paraded before the judge to illustrate the beauty and rarity of plants that fetch thousands of pounds on the black market. In sentencing Henry Azadehdel to a year in prison for smuggling and dealing in endangered orchids, the judge made it clear that the law intends to protect all endangered species, including plants. "The destruction of rare species is not caused by over-enthusiastic collectors but by cynical and ruthless commercial exploitation and trafficking for profit", he said. "If ever a trade wants discouraging, it's this", he added, a sentiment that will be cheered by conservationists the world over.

Azadehdel was caught red-handed, returning through Heathrow from Ecuador just before Christmas in 1987. An astute customs officer confiscated his suitcase full of green shoots and called in the experts to identify them. The plants were taken to the Royal Botanical Gardens at Kew, where botanists identified them and then held them in a "bonded" greenhouse for Customs and Excise.

At the time Azadehdel walked through customs at the airport the plants were easily identifiable as wild specimens. Plants from a glasshouse have almost unblemished, healthy-looking leaves. Plants torn from the jungle are usually damaged, with broken roots, chewed leaves and sometimes an encrusting covering of lichens and mosses.

Orchids are probably the most spectacular flowers on Earth. From one basic pattern, evolution has sculpted thousands of shapes in every imaginable colour. The International Union for the Conservation of Nature and the World Wide Fund for Nature estimate that of the 60,000 species of organisms that will become extinct in the lifetime of a child today, one in ten will be an orchid. One group, the primitive slipper orchids, is at special risk.

At least half of the 70 species of slipper orchid from tropical Asia are seriously threatened in the wild. In many cases the threat comes from the destruction of their habitat. For some species, however, the biggest threat is from the trade that

flourishes to supply collectors with a taste for the weird and wonderful. Collectors, prepared to pay thousands of dollars for a single specimen of a rare orchid, have encouraged the development of a much larger trade. The irony is that as dealers strip orchids from the wild, the prices fall and the trade spreads beyond the rich collector to the enthusiast with a greenhouse. Specimens that fetched hundreds of dollars ten years ago now sell for as little as $5.

Until recently the extent of the trade was obvious from the pages of advertisements in magazines for the orchid grower. Dealers advertised the fact that their specimens were "jungle collected", even though trade in many of the species was illegal. Now banned from offering such plants, dealers simply list the species and country of origin signalling that the specimen has come from the wild.

Most orchids on sale today are raised in glasshouses. They are either hybrids, or pure species raised from seed. This is the legitimate side of the business and does not threaten wild species. Indeed, most orchid nurserymen are keen to eliminate the illegal trade. While they spend years developing better ways of rearing glasshouse specimens, the illegal traders undercut their prices and damage their business.

Despite successes in rearing some of the rarest species, there is a tiny proportion of collectors who still want the authentic plant, plucked from the jungle.

Trade in wild orchids has increased tenfold in the past five years. The biggest markets are Japan and the US, but there is a thriving trade in Europe and Australia and even in the tropics, in Singapore, Malaysia and Mexico. Japanese collectors pay the highest prices for the rarest plants.

Almost as soon as a new species is discovered, unscrupulous dealers seek it out, often quizzing local people and offering a few cents for each plant. The clever dealers scour the academic journals where botanists publish their research in an effort to piece together the habits and whereabouts of a new species. The rewards are so great that some dealers can afford to mount expensive expeditions worthy of the largest botanical institution. Once they have found what they are looking for, they may even clear out every specimen to prevent their competitors from offering the same species.

Rare plants are subject to the same legal protection as rare animals. Most countries now have laws on trade in endangered species. Internationally, the trade is regulated by the Convention on International Trade in Endangered Species (CITES). The treaty came into effect in 1975 and almost 100 countries have ratified it. CITES seems to have helped to stem the trade in endangered animals but it has not worked so well for plants. The treaty virtually bans all movement of the most endangered species, those listed in Appendix I, if they are collected from the wild. Species listed in Appendix 2 can be exported, but each plant needs an export permit. The European Community goes further and demands an import permit too. In each country an organization, usually a government department, is responsible for issuing permits and for checking on the legality of the export and import. In the UK, for example, the Department of the Environment is responsible.

In many countries where the rarest orchids grow, CITES has failed to control trade in endangered species. Many countries have the machinery in place to carry through the procedures involved, yet lack the specialized knowledge to identify the specimens. Where corruption is commonplace, specialist knowledge can be harmful, providing the confirmation a corrupt official needs to ask a high price for a plant.

Even without corruption it is all too easy for dealers to acquire the documents they need to carry on their trade. They can simply claim that their plants were raised in nurseries or that they are hybrids. Unless CITES officials have a detailed inventory of who grows what in nurseries, or can identify a rare species from small green shoots, they stand little chance of proving otherwise. The trade in propagated plants is massive and legitimate. It provides a good cover for the illegal trade.

Illegal dealers show remarkable cunning. In countries that forbid the export of orchids collected from the wild, they simply smuggle them out of the country and acquire the appropriate documents elsewhere. For instance, plants from Burma, which has strict controls on exports of orchids, appear on the market as exports from Hong Kong and Thailand.

The trade operates at several levels. At the bottom are the local people who collect the plants, earning a few cents per plant, perhaps $5 for a particularly rare species. These collectors may be hired directly by the big dealers or they may collect for local middlemen. In the Far East it is easy to buy wild specimens of rare orchids in local nurseries. Sellers may recommend that you wrap your purchase in your underwear to prevent detection at customs but are otherwise unabashed, well aware of the extra value of jungle collected orchids. Even in the Far East, however, the law is beginning to tighten its grip. In April, the Ministry of Agriculture in Hong Kong seized almost 7,000 Chinese slipper orchids destined for sale in the FRG. The dealer was fined and lost plants worth HK$350,000.

Many conservationists claim that the only way to enforce the terms of CITES is at the point of import. If plants are refused entry, the market should dry up. Another way to slow the trade is to destroy the attraction of rare species.

The saddest side of this story is that there is no need to drive any wild orchid to extinction. Almost every one of these species can be propagated from seed and raised in nurseries. Plants grown in nurseries are usually healthier and better formed. Plants taken from the wild usually suffer in the process. Collectors, rarely dealers themselves, tear plants from trees or cliffs, damaging roots in the process. Stuffed in bags, the plants are often on the road for many weeks and arrive battered and dehydrated.

As nurseries and botanic gardens become better at propagating rare species from seed, they may be able to flood the market with specimens. As the price falls, illegal traders should find that it is no longer worth the risk or effort of tracking down wild plants. (Extracted from New Scientist, 24 June 1989)

ATCC news - 14 new molecularly cloned viruses and viroids added to ATCC Plant Virus Collection

Clones to the following viruses are available:

(1) Cauliflower mosaic virus (two clones); ATCC Nos. 45031 and 45032;

(2) Tobacco etch virus (two clones); ATCC Nos. 45035 and 45036;

(3) Beet curly top virus; ATCC No. 45037;

(4) Brome mosaic virus; ATCC No. 45038;

(5) Cassava latent virus; ATCC No. 45039;

(6) Tobacco mosaic virus; ATCC No. 45045.

Also available is a new supplemental listing to the ATCC Animal and Plant Virus Reference Catalogue entitled Plant Viruses and Antisera, November 1988. This supplement lists 135 viruses and viroids and 65 antisera. Direct enquiries to: ATCC MKTING NR. 26, 12301 Parklawn Dr., Rockville, MD, 20852, USA. (Source: AEA Bulletin, Vol. 4, No. 2, April 1989)

AIDS compared to Black Plague, seen worsening

By the turn of the century, "AIDS will have made the Black Plague look like a Sunday School picnic", the corporate spokesman for a newly public Portland-based biotechnology company, Biogan Medical International, told an assembly of investors.

"If the present trend of infection continues as expected", Jonathan Mann, director of the world Health Organization's Global AIDS programme, has said, "AIDS will have wiped out the population of Africa by the year 2000 and have become well entrenched in the other continents. This forecast presumes no vaccine and no cure, neither of which is in sight."

"While the comparison with the Black Death may appear dramatic", L. William Glazier, vice president of corporate relations of Biogan Medical International, says "it is not exaggerated when one considers that the 14th-century plague was confined to the Eurasian land mass and eliminated "only" a third of Europe's population.

"To the great misfortune of modern man, this new plague has struck in the air age, with daily travel among the nations. Its incidence appears to be in direct relation to the presence of foreign carriers, as evidenced by Switzerland, now experiencing the highest incidence of AIDS in Europe. But even relatively closed societies like the Soviet Union and China have been contaminated."

Mr. Glazier says he was quoting "previously published if insufficiently disseminated forecasts", primarily from the annual World Health Organization conference on AIDS held in June of 1988 in Stockholm.

Recent evidence indicates, as recently testified to by United States Surgeon General Koop, that the infected can harbour the virus for a decade or more, without symptoms, before finally testing HIV positive. "Equally alarming", Mr. Glazier told the Biogan Medical investors, "is the recent confirmation of the virus being identified in the other body fluids, i.e. saliva, tears, urine, and spinal fluid, coupled with the rapid mutation of the virus.

"This information has raised the question in some circles as to the possible spread of the virus through means other than of semen or blood.

Should that become a reality, the spread of the disease would multiply by literally thousands of times over what we know today - a theory no one wants to even contemplate due to the disastrous consequences that would result."

The Biogan Medical International executive said that while there has been no evidence of transmission by casual contact in the 1980's, "the appearance of new and deadlier strains in apparently all the bodily fluids presents ominous implications for the 1990's and beyond". (Extracted from Chemical Marketing Reporter, 17 April 1989)

"Cancer virus" contaminates the USA's blood banks

A retrovirus that brings about leukaemia in adults is found ten times as often in US blood supplies as the AIDS virus. The retrovirus is still rare in blood banks, but it poses a risk to recipients, say researchers at Johns Hopkins University in Baltimore, Maryland.

The virus, human T cell lymphotropic virus 1 (HTLV-I), infects the white blood cells. In 1 to 3 per cent of carriers, it goes on to cause cancer, according to medical researchers. It is thought to be related to the human immunodeficiency virus (HIV I), which causes AIDS.

The American Red Cross began screening blood supplies for HTLV I in December 1988. The subjects for the study in Baltimore were tested earlier. To date, no one has reported a case of T cell leukaemia contracted from a blood transfusion. Kenrad Nelson, director of the study, however, notes that most cancers, including leukaemia "may take decades" to arise from infection with HTLV I.

Like HIV, HTLV I can also be spread sexually, through contaminated needles, or from an infected mother to her infant. Screening tests for HIV do not recognize antibodies to HTLV I. It was thought that the HIV test would still serve as a "surrogate" test for HTLV I, notes Nelson, because risk behaviours and means of transmitting the two viruses are very similar.

A problem with testing for HIV arises from the lag of several weeks or months after infection before antibodies to the virus first appear in blood. The same problem exists with HTLV I: the so called seroconversion takes up to 12 weeks.

The team at Hopkins also notes that preparations of platelets may be infected with HTLV I more often than other blood products. White blood cells, which are especially concentrated in these preparations, are the virus's principal target.

"Our findings suggest that there is a very real risk of HTLV I infection by this means that is greater than the risk of HIV I", the researchers write in the New England Journal of Medicine (4 May 1989). The results, they add, underscore the risks associated with transfusion and should "impel

further efforts" to limit the use of blood components to cases when it is unavoidable. (Source: New Scientist, 13 May 1989)

Ecological Society of America reaches conclusions on deliberate release

The US Industrial Biotechnology Association (IBA) has enthusiastically embraced the conclusions of a report on environmental releases of genetically engineered organisms prepared by the Ecological Society of America.

The IBA points out that the report is the third in recent years to conclude that: "Careful design of transgenic organisms, along with proper planning and regulatory oversight, will ensure that these new organisms will pose little or no ecological risk".

The report, published in Ecology (Vol. 70, No. 2, April 1989), concludes that: "genetically engineered organisms should be evaluated and regulated according to their biological properties, rather than according to the genetic techniques used to produce them". It also notes that: "Case-by-case review is currently the most scientifically sound regulatory approach because of the diversity of products that can be developed and the complexity of predicting their ecological fate". (Source: Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

Mapping the human genome

The project to map the human genome is still in its infancy. No one knows how scientists will tackle the exercise, what they will find or, perhaps most importantly, how they will apply the knowledge they gain. These three facets of the project are intricately linked, not only to each other, but also to myriad legal and ethical issues.

To discuss the ramifications of knowing more about the human genome, a small group of scientists, philosophers and specialists in law, ethics and theology met in Bern, Switzerland. The symposium, entitled Human Genetic Information: Science, Law and Ethics, was organized by the Ciba Foundation, in celebration of its 40th anniversary, together with the Academic Commission of the University of Bern.

The most immediate problem facing scientists is how to go about determining the sequence of the base pairs that make up the genetic code. James Watson, the director of the American programme to map the human genome and co discoverer of the double helix, has suggested that individual countries should take responsibility for particular chromosomes. Some scientists at the meeting strongly disagreed, however.

The mapping of the human genome will undoubtedly take more than a generation to complete. Even if a machine were available that were capable of producing, in one day, the sequence of a strand of DNA containing a million pairs of nucleotides, then it would still take about 20 years to sequence both strands of the human genome.

When Governments are contemplating spending millions of dollars on the project, the question of whether it is practicable to sequence the entire genome now is bound to come to the fore. Some researchers at the symposium warned of the dangers of putting too much emphasis on the benefits that the project would have for medicine: basic biology would also profit, they said. But several others said they had no doubts that the justification for the work would be the medical benefits.

One immediate gain will be the identification of genes responsible for many inherited diseases. There are several thousand human diseases caused by mutation of a single gene, which are inherited in a simple Mendelian fashion. These so-called monogenic diseases include, for example, thalassaemia, cystic fibrosis, Huntington's disease and Duchenne muscular dystrophy. In most of these cases, scientists do not know which gene has mutated, or what its normal product is.

Once that information is available, doctors will be able to offer tests to couples planning to have children, to determine whether they both carry a recessive gene for a disease such as cystic fibrosis, for example. Prenatal diagnosis will also be available for a wide range of genetic diseases. In the case of monogenic diseases that do not become apparent until late in adult life, there will be simple tests available to tell people in affected families whether they carry the mutant gene.

The business of prediction will be more complicated for most other diseases that affect adults, such as coronary heart disease, cancer, diabetes, arthritis and mental illness. There is often a genetic component to these "polygenic" diseases, but they are not inherited in a simple and predictable way. In addition, environmental factors such as diet, smoking and lack of exercise may play a role, or even be entirely to blame for such diseases.

Nevertheless, it may be possible to define the increased risk that individuals suffer by virtue of their genetic make up.

The wider availability of techniques to diagnose diseases with a genetic component will raise difficult legal and ethical issues. Diana Brahams, a specialist in the law relating to medicine, said it might be possible in future to identify people likely to develop a serious disease, long before the symptoms appear.

Such information could be "of considerable interest and value to any prospective employer, insurer, marriage partner or family member and would be of serious concern to the individual", she said. People will have to tackle the question of how far sensitive genetic information should be made available to the patient, or to other interested parties.

People probably harbour much greater fears about how scientists could use the information they obtain about the human genome for treatment, rather than diagnosis. Once geneticists know which genes are responsible for monogenic diseases, the prospect looms of being able to carry out some kind of gene therapy. One way to do this would be to introduce a normal copy of the faulty gene into the cells of the body, or somata.

While this approach might work for cells that can function anywhere in the body cells that produce hormones might be one example it would not help with most inherited diseases. In cystic fibrosis, for example, the defective gene alters the

function of membranes in many different organs, from the lungs to the sweat glands.

For such monogenic diseases, the alternative would be to insert the copy of the normal gene into the fertilized egg (zygote), following in vitro fertilization. Once the embryo was back in the uterus, all its cells would have a copy of the normal gene and, according to theory, be capable of functioning normally.

Charles Weissmann, of the Institute for Molecular Biology in Zurich, Switzerland, pointed out that even if the technology to perform such gene therapy successfully were available, it would first be necessary to establish whether the zygote carried the defective gene. Rather than proceeding with gene therapy on such zygotes, it would be much simpler, he said, just to replace unaffected embryos in the woman's uterus.

The question of who should regulate the application of the new-found knowledge about the human genome is a taxing one. Brahams pointed out that the law tends to respond to past events. Overregulation would suffocate research but, "on the other hand, if you are too liberal, people can do unpleasant experiments", as history has shown. There was clear concern among scientists at the meeting that inflexible laws would interfere with research. There was a high measure of agreement about the desirable ways of regulating the research. Governments should set up regulatory agencies (similar to the Voluntary Licensing Authority which oversees embryo research in the UK) that could monitor research and react swiftly to new discoveries.

Much of the unease about the future scope of genetic manipulation may have its roots in ignorance. Much needs to be done with regard to the education of journalists, members of parliament, lawyers and religious leaders and, of course, the public at large. (Extracted from New Scientist, 8 July 1989)

Genome's tortuous path

The Human Genome Mapping Organisation (HUGO), an agency with the task of co ordinating international research to map human genes, was launched in Geneva in May 1989. HUGO is asking scientific funding agencies for money to pay for fellowships, scientific meetings, and to enable it to advise Governments on the "scientific, ethical, social, legal, and commercial implications" of genetic research.

HUGO says that a map of human genes "should provide a basis for preventing or treating most human chronic disease". (Extracted from New Scientist, 6 May 1989)

DNA fingerprinting

The application of DNA fingerprinting to forensic samples is gaining rapid acceptance in the US criminal court system. In less than two years, DNA data have been considered as evidence in more than 80 criminal rape and murder trials in 27 states, leading to at least 64 convictions or pleas of guilty by the defendants. But some biologists and legal experts have expressed concern that the evidence is not as infallible as judges and juries are being led to believe.

The power of the technique is not in dispute. When performed correctly, it can match DNA from an individual to that extracted from samples of blood, hair or semen left at the scene of a crime. The most commonly used method relies on restriction fragment length polymorphisms (RFLPs), regions of DNA that vary among individuals, producing fragments of different sizes when the DNA is cut with restriction enzymes and run on sizing gels. When probes for a large enough number of RFLPs are used, the likelihood of a match between two unrelated individuals may be less than one in 100 million.

Forensic DNA testing is at present carried out by three commercial US laboratories, and the Federal Bureau of Investigation (FBI).

Concern about the tests focuses on the potential for human error in sample treatment or data analysis, the interpretation of DNA patterns, the uniformity of criteria used to determine whether two samples match and the population studies on which the predicted likelihood of a mismatch is based.

In written testimony presented to Congress during hearings on DNA fingerprinting in March, law professor Barry Scheck, a member of the New York Governor's Commission on Forensic DNA Typing, writes that in many of the court cases there has been "little, if any, informed cross examination of private DNA vendors, and few qualified expert witnesses testifying in opposition. The defence lawyers in these cases ... have been overwhelmed."

That trend may be changing, as the defence in a murder case under way in the Bronx has lined up an impressive string of biologists to testify to the potential weaknesses in the analysis of DNA extracted from a blood spot on the defendant's watch. The witnesses included human geneticists Eric Lander and David Page of the Whitehead Institute, Conrad Gilliam of Columbia University and Howard Cooke of the Medical Research Council in Edinburgh.

Among the issues raised in the case was the lack of adequate controls in the DNA analysis performed by Lifecodes Corporation, a New York company specializing in DNA fingerprinting.

The strength of DNA fingerprinting data lies in the potentially low probability of a match occurring between two unrelated samples. But Lander warns of a potential error that could arise in the reporting of those probabilities, if standards for a match are not uniform. The stringency of the standards for what constitutes a match determines the number of identifiably distinct variants of a given RFLP that exist in the population, and that, in turn affects the probability that a match might occur at random.

There is no set of guidelines to help individual testing laboratories to make decisions about matching criteria and other procedures, nor is there a standard proficiency testing or licensing process for laboratories that perform the DNA analysis.

An independently conducted blind trial of commercial laboratories was carried out in 1987 by the California Association of Crime Laboratory Directors. Although the laboratories knew they were under investigation, a worrying number of errors was revealed.

infantile diarrhoea. With the new agreement, the research team will receive financial support from SANCOR for the scaling up of the laboratory procedure. The commercial firm was chosen among others in a public bid by CONICET to carry out the development programme.

During the signing of this agreement, Mr. Oreste J. Manrique, president of SANCOR, pointed out that his company had also signed another agreement with the University of Buenos Aires a few months previously. At that time, the type of research involved was the development of new technological procedures for the production and commercialization of milk. These events, together with important investments made by SANCOR during the last years, would allow the company to be present, not only in the domestic market but to compete successfully with high quality products in the international market.

Regarding the clinical assays of the milk obtained by the CERELA fermentation process these were done at the Jesus Child Hospital (Tucumán City) in affected children with chronic or acute diarrhoea. The level of success was 91 per cent of all treated cases. Many of these children had severe malnutrition (58 per cent) and some of them (7.7 per cent) more severe symptoms. In all cases the putative agent was eliminated and there was no need for new hospitalization.

Lactobacillus present in the CERELA fermented milk colonized the intestinal flora which in the affected children was unbalanced due to malnutrition and constant attack from environmental agents. This colonization, in turn, amplified the "barrier effect", a mechanism that represents the organism contention barrier to aggressive external bacteria, with the concomitant elimination of harmful micro-organisms.

Together with nutrition and intestinal protection it is important to mention the enhanced activity of the immune response provoked by the lactic bacteria in the CERELA fermented milk. This kind of activity is probably due to an increase in the number of macrophages induced by those kinds of bacteria. (Source: Bulletin of the Argentine Forum of Biotechnology, Vol. 2(4):9-10, 1989, Buenos Aires, Argentina)

**Austria**

Biostimulation by photons

Austrian research into biophotons has proved a differentiated sensibility of cells to photon emissions. By applying specific wavelengths representing the photon emission of oxygen molecules to various cells (He-La-cells, human stem cells), the Atominstitut of the Austrian Universities has found that photons of 630 nm and 760 nm induce cell division, whereas photons of 1,060 nm seem to suppress cell division.

Research was based on the study of human phagocytes (leukocytes, monocytes, macrophages) which emit photons of low intensity in the visible and near infrared region during immune defence. The wavelength are typical for bands of excited singlet oxygen molecules normally involved in phagocytosis. Practical use of biostimulation is seen in immune defence and in accelerating cell growth in bioreactors. (Source: BIO Technica Journal, No. 2)

New commercial set up

Four Austrian major companies are joining forces to boost the country's efforts in biotechnology research. OLAG will be the major shareholder in the company called Biotechnologische Forschungsgesellschaft (BTF), with a 22 per cent share. Chemi-Holding, OMV and VAIG will have equal shares in the remainder.

Currently, Austria spends about Sch 19 million ($1.5 million) per annum on biotechnology. (Source: Manufacturing Chemist, April 1989)

**Australia**

Western Australia biotechnology park

Coogee, south of Perth, will be the site for a planned biotechnology park which will process wastes from industries in the area using biotechnologies. Many of these are animal and fish derived and the aim is to add value to the wastes and establish new export industries. The Technology and Industry Development Authority (TIDA) recently called for expressions of interest from consultants to carry out more detailed feasibility studies. Over 80 expressions of interest were received and 16 submissions. A consultant is expected to be appointed in the near future.

The consultant's study will not only look at the techno-economics of different processes to treat the wastes, but will also look at market size, competition and exporting of proposed products. Further information on the proposal can be obtained from Dr. Sue Meek at TIDA. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

Australia's first recombinant release goes commercial

The first commercial release of the new-strain Agrobacterium radiobacter K1026 spells a long-awaited victory of the bacterial disease crown gall - the damnation of orchardists and nurserymen all over the world. Recently registered in New South Wales, and already being marketed from there (under the brandname Nogall(R)) by sole-distributers Bio-Care Technology Pty Limited, the new strain is the result of years of research and construction by Professor Allen Kerr and his team of D.A. Jones, M.H. Ryder and B.G. Clare, in co-operation with Dr. Stephen Farrand from the University of Illinois. The pathogen causing crown gall inhabits many soils and it manifests itself mainly on stone fruit and nut crops, along with some ornamentals such as roses. Rootstocks and stems of bacteria-infected plants develop hard woody galls which serve to diminish the affected plant's wellbeing by disrupting its intake of food and water - whilst also providing an entry point for secondary infections.

A natural bio-control organism, Nogall(R) K1026 is genetically engineered and not a chemical, herbicide or pesticide. It has been designed to treat the cuttings of documented hosts consisting mainly of the stone fruits (peach, apricot, nectarine, plum and cherry), nuts (pecan, walnut, almond), roses, clematis, persimmon and caneberries such as raspberry, boysenberry etc. While Australia has a gross annual production value of fruit and nuts of something near the $100 million mark and there is a significant crop loss in the first few

years because of crown gall, the importance of Nogall(R) on the world scene is indeed immense.

Treatment is carried out by the application of a liquid slurry of the live Nogall(R) K1026 culture - usually by immersing the seedlings, seeds, cuttings or plant root systems before planting out. A 250-gram pack of Nogall(R) inoculant makes 12 litres of dipping solution - sufficient for a great number of cuttings. This affords long-term protection against a very damaging disease at the cost of just a few cents per plant. Adelaide University's commercial arm, Luminus Pty Limited, hold the patent for the new strain K1026 and the sole marketing rights have been granted to Sydney-based Bio-Care Technology Pty Limited, C/- P.O. Box 367, Woy Woy - themselves long-involved in the manufacture and distribution of K84 throughout Australia. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

International Depositary Authority in Australia to expand range of organisms

The Australian Government Analytical Laboratories in Sydney, which act as an International Depositary Authority under the Budapest Treaty, intends to expand the range of organisms that it will accept. A submission to WIPO will shortly be made regarding acceptance of plant, algal, and animal cell lines, including hybridomas. However, organisms requiring WHO containment class 3 will not be accepted. Furthermore, AGAL will accept deposits in their laboratories in Melbourne and Adelaide, as well as in Sydney. Further details are available from Dr. Ken Newton (Tel: (02) 449 0111). (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

**Brazil**

Santa Catarina Centre for Biotechnology Development

Readers of Issue No. 24 of the Monitor may have noticed the article on the new biotechnology centre at Santa Catarina. Dr. Walter Borzani, the Scientific Director of the Centre, has asked that we inform you of the work the Centre is presently involved in:

1. The Centre is working on four projects:

   1.1 Lactic fermentation of whey.

   1.2 Lysine production.

   1.3 Riboflavine production.

   1.4 Inulinase production.

2. FINEP (a Brazilian agency that finances studies and projects) will provide $US 2 million.

3. Dr. Mario Cesar Cubas is the Administrative Director. Dr. Borzani may be contacted at the following address: Instituto Mauá de Tecnologia, Estrada das Lágrimas, 2035, 09580 - São Caetano do Sul - SP - Brazil.

**Canada**

Canadian biotechnology ready to look overseas

At this stage, and with the single European market looming, Canada is turning its attention from its almost exclusive focus on the North American market. The Canadians feel the time is ripe for its domestic companies to position themselves strategically in order to be able to take advantage of what they see as a European market set to expand at a faster rate than anywhere else in the world.

They also see an opportunity to prepare for increased global competition and to minimize the effect of what could possibly become "Fortress Europe". This is likely to involve establishing a corporate presence within the EC or forming strategic alliances and joint ventures with companies within the Community.

At the same time, Canada feels that in the case of biotechnology it has a great deal to offer foreign companies and wishes to attract European firms to Canada. The main catch being used to achieve this end is the recently signed free trade agreement established between itself and the US.

It is suggested that foreign firms wishing to establish themselves in Canada should use this particular agreement to penetrate a US market which currently represents some 270 million people.

At the same time, by joining with foreign companies in their own country, the Canadians hope to withstand what could potentially become a deep and widespread penetration of their own domestic market by US biotechnology companies.

Spurred on by the government's backing, which includes amongst other measures the establishment, in 1983, of a National Biotechnology Advisory Committee as part of the national biotechnology strategy, Canada's biotechnology sector has developed both scientifically and commercially.

There are now 218 companies involved in the area. Roughly, companies in the country's western regions focus on agriculture and aquaculture, while in the east they are more specifically involved in sea-related technologies. The majority - some 84 - are based in the state of Ontario. Next, comes Quebec with 49 companies, then British Columbia with 34, 13 in Alberta, 9 in Saskatchewan, and 6 in Manitoba. The remainder are scattered in the eastern area.

About one third of the companies involved in biotechnology have a primary interest in the health care sector.

Forty-five per cent of private R&D expenditure in biotechnology is focused on health care, 27 per cent in agriculture, 8 per cent in waste processing, 7 per cent in chemicals/energy, 4 per cent in food/beverage, with the balance shared amongst agriculture, forestry, mineral resources and other areas. Collectively, health care biotechnology firms spend about $27 million/year on R&D. This represents 41 per cent of commercial biotechnology R&D expenditures in Canada. These companies also employ 280 researchers, or 40 per cent of all biotechnology industry research personnel.

Canada's pharmaceutical industry is committed to a doubling of its R&D expenditure between now and 1996. Following the introduction in December 1987 of a law which extended protection for new patents from 4 to 10 years, the industry has promised to devote 10 per cent of its sales up to 1996 to R&D. This compares with the current level of 4.9 per cent or $Can 1.4 billion.

Beside the free trade area formed with the United States, Canada is among the ten largest

pharmaceutical consuming nations in the world, has plentiful and cheap energy and other resources and a GDP growth rate that is one of the higest among OECD countries. (Source: European Chemical News, 12 June 1989)

**China**

Biotechnology venture, a first, entered by US firm and PRC

Cell Technology Inc. has signed an agreement in principle with the Chinese Medical Academy of Science for the development of its "ImuVert" biological response modifier (BRM) anti-cancer drug. The company believes this to be one of the first joint ventures in biotechnc··· initiated with the Chinese Government.

Cell Technology says the agreement is broad, and also allows for clinical trials in China in other tumour models. The company is also exploring economic collaboration which may lead to a joint manufacturing facility in China.

Also under consideration is the possibility of licensing several of the BRM products developed by the Chinese Cancer Institute. These, says Cell Technology, are currently in advanced human clinical trials in China for the treatment of various cancers. (Source: Chemical Marketing Reporter, 3 April 1989)

**Denmark**

Genetic engineering law to be less rigid

Denmark's Minister of Environment is poised to allow the country's first deliberate releases of genetically modified organisms. Following a parliamentary debate on proposed field trials of sugar beet containing either a herbicide-resistant or a virus-resistant gene, Ms. Lone Dybkjaer has indicated that she will grant the approvals that are necessary under the Environment and Gene Technology Act, Europe's only specific legislation for gene experiments. That decision is the latest sign that Denmark's three-year-old law is not necessarily as restrictive in practice as it seems on paper.

Criticisms of the law are only somewhat tempered by the increasing speed with which National Food Agency civil servants are dealing with applications, and by some amendments to the law that came into force on 1 July.

The two genetically modified sugar beets to be field tested in Denmark next year will be the first using a commercially relevant line. One modification makes beet resistant to glyphosate, the active ingredient of Monsanto's herbicide Roundup, which is considered to be more environment-friendly than most alternatives but is toxic to ordinary sugar beet. The other modification is designed to confer resistance to rhizomania, a disease caused by the beet necrotic yellow vein virus.

Denmark's gene law is due for additional amendments in 1990/91. One possibility is that it will at that stage fall into line with European Community regulations. Current Danish law is still considerably more restrictive on the contained use of genetically modified organisms than what seems likely to become the minimum European law.

As for deliberate release, Europe is still in disarray, with current Danish law and German intentions appearing to be the most restrictive. (Extracted from Nature, Vol. 339, 29 June 1989)

Denmark gives green light to interleukin-2

Denmark has granted marketing approval to Cetus Corp's anticancer drug, interleukin-2, to treat advanced kidney cancer. The US biotechnology firm has also received US patents covering Macrophage-colony stimulating factor (MCSF) and PBG M-CSF.

The Danish decision follows the recommendation from the EC's committee for proprietary medicinal products. Cetus intends to sell the drug directly in some EC nations, and has set up marketing operations in France, Italy, Spain, Federal Republic of Germany and the United Kingdom.

Cetus is waiting for US approval as a kidney cancer treatment, and hopes to receive the green light within 12 months. Getting the first approval is a key step as both US and EC regulations allow a drug to be prescribed for any ailment once it has been approved for one indication.

Switzerland's Hoffmann-La Roche stands to benefit from the Danish decision. Under a deal signed at the end of last year, Cetus and Roche averted a protracted patents battle by signing a cross-licensing agreement.

M-CSF stimulates production of white blood cells which play an important role in the body's defences. Clinical animal studies suggest the protein may be useful in wound healing and restoring white cell populations. (Source: European Chemical News, 17 July 1989)

**European Community**

Engineered organisms' guidelines take shape

European Community (EC) environment ministers on 8 June agreed on guidelines for the use of genetically modified organisms in laboratories or production facilities, but disagreed on how to regulate their release into the environment.

The guidelines were proposed last year by the European Commission, the executive body of the EC, and later amended by the European Parliament. The meeting of the Council of Environment Ministers was a first response to the amendments suggested by the Parliament, which held a second reading of the proposal this autumn. The council has the final say on the content of the proposal.

The Council, meeting in Luxembourg, adopted some of the stricter guidelines proposed by the European Parliament on the use of genetically modified organisms in laboratories and production facilities. Under the guidelines adopted, "dangerous" modified organisms must be licensed by national authorities the first time they are used in a laboratory and every time they are used for production. In addition, the council recommended flexible deadlines for licensing procedures in order to allow for public participation.

The final proposal reflects FRG wishes for stricter regulation, which France and the UK have traditionally opposed. The ministers disagreed about whether to require Community-wide licensing of products containing modified organisms and intended for release. The minister from Spain, which holds the EC presidency until next month, decided after a brief discussion to return the proposal to a ministerial committee for further debate.

Regulations approved by the Council comprise a minimum standard for EC member States, which are

bound to adopt them. Member States are free to add to Community regulations. (Source: Nature, Vol. 339, 29 June 1989)

CEFIC biotechnology move

The European Chemical Industry Federation (CEFIC) has set up a top level group to advise on the application of biotechnology.

The group, headed by ICI director Dr. P. Boyle, is to formulate policy rather than act as a political lobbyist. Its aim is to ensure a competitive position in biotechnology for European industry.

Other senior advisory group members are main board directors of Monsanto, Sandoz, Montedison, Rhône-Poulenc and Hoechst. (Source: European Chemical News, 24 July 1989)

EC genome project goes on ice

The new European Commissioner for Research and Development, Mr. Filipo Maria Pandolfi, has frozen an ECU 15 million (9.75 million pounds sterling) human genome research project designed to map the human genetic code as a first step towards predicting the likelihood of any individual being afflicted with a particular hereditary disease.

Following pressure from left-wing and Green Euro-MPs and from Christian groups, the project has been shelved until Community-wide ethical guidelines have been agreed to control the uses of biotechnology.

The decision may be unfortunate, but the demand for Community-wide ethical guidelines covering such research is understandable. There is inevitable concern that the research could pave the way for human genetic engineering along the lines advanced in the "eugenics" theory embraced by the Nazis. Even if the research "only" results in couples being able to increase their chances of having "normal children" or to choose the sex of their children, then there are certainly ethical questions which need to be addressed.

So, while many biotechnologists and scientists will view this latest development with intense frustration, they should recognize that it is symptomatic of the genetic engineering-related concerns that will surface during the 1990s in the public mind. Europe's scientists should respond now and engage in a much more active and open debate with the public and its representatives about the world they are helping to create. (Source: Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

Biotechnology in EUREKA projects

At the invitation of the Austrian Government, Ministers from 19 European countries and the Vice-President of the EC Commission met in Vienna on 18 and 19 June 1989 for the 7th EUREKA Ministerial Conference.

At the Conference 89 new projects were announced with an estimated value of 1,600 million ECUs, bringing the number of approved EUREKA projects to 297, with a total estimated value of over 6,400 million ECUs. At present 1,600 firms and research institutions are engaged in EUREKA projects.

The Conference has added 14 new projects in biotechnology to the 41 projects (434 million ECU) under way in this field. They are covering diagnosis and treatment of diseases, genetic engineering of plants and biotechnological production processes.

From the previous total of 41, 27 projects cover both clinical and diagnostic applications touching upon a range of diseases. Two projects involve R&D on cancer detection and treatment. Three deal with sexually transmitted diseases, and others aim to develop malaria vaccine, advanced diagnosis and treatment of diabetes, allergies and high blood pressure.

Further work is in progress on a variety of different medical aspects such as:

- An expert system for health examination;
- The electronic identification of blood bags;
- The functional restoration of the ability to walk by implanted neurostimulation;
- Bio-medical sensors; and
- New biocompatible ceramics.

There were 12 projects in the agro-biotech area, some involving genetic engineering to improve the quality and disease resistance of plants such as the sunflower, the tomato and corn. Others deal with the production of growth promoters and natural flavours. Last but not least, work should be mentioned on the development of new sparkling beverages.

Eight of the biotechnology projects have primarily a production methods orientation. Several of these projects centre on process applications, such as the high volume production of animal and/or human cell cultures, antigen marking and filtration/separation techniques. Another project deals with separation processes functioning under zero gravity conditions (e.g. as in space), another with an automated and programmable laboratory for work with DNA (analysis, hybridization, cloning, sequencing, etc.). (Source: BIO Technica Journal, No. 2)

EC programmes and directives

**EC programme ECLAIR**

To improve the interfaces of agriculture and industry the EC Commission will support pre-competitive research by the ECLAIR programme (European Collaborative Linkage of Agriculture and Industry through Research). The programme funded at 80 million ECU covers three broad themes: candidate species for agriculture, extraction/transformation of agricultural products for industry, and integrated agricultural/industrial systems. After a general agreement in the Council, a first call for proposals was published in the EC Official Journal No. C 324 on 17 December 1988. (Code 89/1)

**EC programme FLAIR**

The EC Commission has proposed to the Council a R&D programme in food science and technology called FLAIR (Food-Linked Agro-Industrial Research) for the time period 1989 to mid 1993. The programme will support R&D in the assessment and enhancement of food quality, in food hygiene, safety and toxicological aspects, and in nutrition and wholesomeness aspects. The programme still to be adopted by the Council earmarks 25 million ECU for concerted actions, cost-shared projects (up to 50 per cent support), training and mobility grants. (Code 89/2)

**EC programme BRIDGE**

The EC Commission prepared a research and training programme for biotechnology called BRIDGE (Biotechnology Research for Innovation, Development and Growth in Europe). It should take in 1990 the succession of the ongoing Biotechnology Action Programme (BAP). The programme will reinforce the existing laboratory network and extend it to high priority areas. It will support information infrastructure, enabling technologies, target oriented research tasks for agricultural and industrial biotechnology, and biological safety assessment. (Code 89/3)

**Directive on risks from biological agents**

The EC Commission has prepared a "Council Directive on the protection of workers from the risk related to exposure to biological agents at work" (COM(88) 165 final). The Directive submitted to the Council for approval says that the risk arising from exposure to biological agents at work must be assessed before workers can be given adequate protection against them. Member states will have to classify biological agents according to their level of danger, using the definitions proposed in this Directive. (Code 89/4)

**Directive on protection of biological inventions**

A "Council Directive on the legal protection of biological inventions" (COM(88) 496 final) has been proposed by the EC Commission to establish harmonized, clear and improved standards for protecting biotechnological inventions. The objective is to systematically adapt existing patent law principles to the field of biotechnology. It covers patentatibility, scope of protection, protection of plant and animal varieties, deposit and access, reversal of the burden of proof, definitions and financial provisions. (Code 89/5)

**Directive on contained use of modified organisms**

In the Meeting of 24/25 November 1988, the EC Council started discussion on the proposed Directive on the contained use of genetically modified micro-organisms (GMMs). It introduced a system of notification of operations, the application of specific containment measures, measures concerning accidents and waste management. The Council also noted the progress of discussions on the proposed Directive on the deliberate release to the environment of genetically modified organisms. Approval of the two Directives is expected in 1989. (Code 89/6) (Source: BIO Technica Journal, No. 1)

**Federal Republic of Germany**

Biotechnology controls approved

The FRG's federal cabinet has passed a bill to regulate genetic engineering techniques used by industry and scientific organizations. It will now go to the Bundesrat, the upper house of parliament, for approval.

Chemical and pharmaceutical companies participating in the long running debate about genetic engineering were rewarded by seeing some of the more restrictive clauses in the bill modified. The fear was the laws would be harsh enough to prompt an exodus of pharmaceutical research and development from the country.

Most significant was the amendment dividing genetic projects into four categories, graded by number from low to high risk. Most projects currently being pursued by the chemical industry (production of human insulin, pro-urokinase, interferon or erythropoetin) will fall into low to moderate risk categories, 1 and 2. Categories 3 and 4 will apply to work with known pathogens or other hazardous substances. Plans for category 1 and 2 projects will not have to be submitted to a public hearing. This complex bureaucratic procedure is currently applied to these projects, as well as category 3 and 4 projects, under the country's emission control laws.

The federal health authority, Bundesgesundheitsamt (BGA) will be responsible for rating projects, but the federal states will also have the power to approve or deny projects in their states.

Companies and laboratories will be required to supply federal states with "all pertinent information" needed to evaluate the safety of their plans. Manufacturers and researchers will also have to prove that they are insured against any risks associated with the technology or the materials used. Fines or up to five years' imprisonment are foreseen for non-compliance with the law's provisions.

Plans for the release of genetically altered micro-organisms or plants into the environment will have to be evaluated by the federal environmental authority, Umweltbundesamt, as well as by the Bundesgesundheitsamt. (Source: European Chemical News, 24 July 1989)

Biotechnology plans held up

Three genetic engineering projects are being held up in the FRG as a result of public hearings, which have to be held under new emissions control legislation.

A public hearing on BASF's plans to build a research centre at Ludwigshafen to test production of the tumour necrosis factor (TNF) drug, has been suspended after opponents to the project claimed BASF failed to provide sufficient information. Dates have yet to be set for hearings on plans by Hoechst subsidiary Behringwerke to build a genetically engineered erythropoietin plant at Marburg and Brünenthal's proposal to construct a unit for genetically produced pro-urokinase at Aachen.

However, the FRG's proposed framework law on genetic engineering could remove the public hearing hurdle for most of the chemical companies involved in the field. The first draft of the new law will limit the hearing requirement to high risk projects. (Source: European Chemical News, 8 May 1989)

Summary of first biotechnology programme

The Federal Ministry for Research and Technology has published its "Programme Report Biotechnology" summarizing the results of the first governmental programme "Applied Biology and Biotechnology 1985 1988".

Within this programme the Ministry's annual R&D expenditures for biotechnology increased from DM 123 million in 1984 to DM 261 million in 1989 summing up to about DM 800 million over the four year period of the programme.

The funding was split up between several support instruments:

- 36 per cent for co-operative research university/industry;
- 33 per cent for institutional research;
- 24 per cent for individual research projects;
- 7 per cent indirect specific support measures.

In total about 2,200 new work places have been created for biotechnology research, including 800 for scientists. About 220 companies participated in co-operative research projects with universities involving only 52 large companies.

Since the early 1980s more than 100 new biotechnology companies have been set up, 34 within the programme period. Total industrial R&D expenditure for biotechnology is now estimated at DM 1 billion annually.

The Programme Report surveys results and experiences of the first government biotechnology programme. A parallel report of the Ministry demonstrates advanced applications of biotechnology as examples of R&D projects funded by the programme. (Source: BIO Technica Journal, No. 2)

Microbial ecology research

The new FRG environmental research and technology programme for 1989 to 1994 now published by the Federal Ministry for Research and Technology will include research into microbial ecology. The main objective is to close methodological gaps and increase understanding in the application of micro-organisms.

Research support will concentrate on: development of methods and measuring equipment, standardization of model systems, detailed analysis of microbial interactions, study into microbial control mechanisms, and development of scenarios for the application and technical realization of microbial ecological safety concepts. (Source: BIO Technica Journal, No. 2)

The flowers that bloom next spring

Forty thousand genetically-engineered pink petunias are to bloom in the Federal Republic of Germany despite the continuing controversy over new legislation to control such experiments. The Federal Health Office approved an experiment by Heinz Saedler of the Max Planck Institute (MPI) for Breeding Research in Cologne, but the MPI group will not plant the petunias until 1990; approval came too late for the 1989 season. Environmentalists have opposed the petunia planting, not because they thought the flowers harmful but because they thought it would set a precedent for releases of other genetically-engineered organisms. (Source: Nature, Vol. 339, 1 June 1989)

Magnetic microspheres for cell separation

The Klinikum of University Kiel, FRG, has developed a simple technology for analytical and preparative cell sorting in heterogeneous cell suspensions using magnetic immunomicrospheres (MIMMS). The principle consists in combining the separation specificity of antibodies o other relevant molecules with the physical separation power of a magnetic field. The principle is effective, simple and fast, non-toxic and solves the problems of quantity in human islet or bone marrow transplantation. (Source: BIO Technica Journal, No. 2)

**Japan**

Human genome sequencing by committee

Last May a sub-committee of the Science Council of Japan, a non-government body directly elected by academics, issued a report recommending a greatly expanded effort regarding the country's human genome project. Two sub-committees of the Ministry of Education, Science and Culture also recently submitted similar recommendations to the Ministry's own science council. Last year the Science and Technology Agency (STA) issued a vaguely-worded call for a project but gave no indication of the direction it should take.

But apart from a small-scale effort at STA's institute of physical and chemical research (RIKEN) to develop automatic DNA sequencing machines, a project started many years ago by Professor Akiyoshi Wada of Tokyo University, there is no project under way in Japan.

Kenichi Matsubara of the Insitute of Molecular and Cellular Biology of Osaka University, head of one of the education ministry's sub-committees, says that even if the Ministry's science council accepts the sub-committee's recommendations when it meets to discuss them, it will be at least two years before government funds will be available to support a project.

Meanwhile, with the council's approval, Matsubara says the sub-committee hopes to launch a "small rocket" using emergency funds that the Ministry sets aside for research on earthquakes and the like. The funds will be used to organize more committees of university researchers and to cope with demands for genome information from overseas by, for example, improving computer programming facilities.

The report from the Science Council of Japan calls for the establishment of an organization to co-ordinate a joint research effort by various government agencies and ministries. This is not an easy task, but nevertheless, Matsubara hopes that by drawing together university researchers with "emergency" funds from the Ministry of Education, an "invisible committee" will be established during the next two years that could co ordinate an inter-agency project.

Matsubara is Japan's representative of the Human Genome Organization (HUGO), established last year to co-ordinate worldwide efforts on the project; he has been trying to raise funds in Japan to support HUGO. (Source: Nature, Vol. 339, 29 June 1989)

Companies agree to participate in drug development

Thirty six Japanese companies have agreed to participate in a programme under the auspices of the Japanese Ministry of Public Welfare to develop drugs to treat AIDS (acquired immunodeficiency syndrome) and AIDS related diseases. The research will focus on five areas: drugs to kill the HIV (human

immunodeficiency virus) directly; drugs to prevent the development of AIDS following infection by HIV; drugs to treat the associated diseases Carinii pneumonia and Karposi's sarcoma; reagents and equipment for diagnosing AIDS; and methods for evaluating the effectiveness of anti-AIDS drugs.

Companies participating in this programme include major pharmaceutical houses and leading biotechnology firms: Chugai Pharmaceuticals (Tokyo), Shionogi Pharmaceuticals (Osaka), Dai-Nippon Ink Chemical Industries (Tokyo), Mitsui Toatsu (Tokyo), Asahi Kasei (Tokyo), and Toray Inc. (Tokyo). The programme budget for next year will be about 43 million yen. (Source: Bio/Technology, Vol. 7, April 1989)

**Jordan**

A report on the current biotechnological activities and research in Jordan

**Royal Scientific Society**

A comprehensive study on the potential of research and development in the field of biotechnology has been conducted to identify the areas of priority in biotechnology that are of utmost importance to the development of Jordan.

Furthermore, to ensure the ideal utilization of the available specialized manpower and facilities, a recommendation was issued to establish a National Biotechnology Centre. The necessary steps to establish the centre were identified by a study conducted by Jordan's Royal Scientific Society in which three stages were developed using the Critical Path Method (CPM) and the Programme Evaluation and Review Technique (PERT). Thus, by using the above two methods the critical path for the centre's development was identified in addition to the start date, finish date and total cost of each step utilizing the two methods and RSS computers to constantly monitor and update the development plan.

In addition to the above activities, a study for the assessment of the environmental risks from biotechnology was conducted in which a Markov chain model was developed for that purpose. The study assesses the potential risks of introducing by mistake or on purpose a genetically altered microbial strain to the environment.

**The Jordanian universities**

In addition to the research activities that were presented at the First Arab Conference on the Perspectives of Modern Biotechnologies in the Arab Countries, a number of research activities on single cell protein and waste utilization have been conducted. These research and development activities are an upgrade from the usual laboratory work to the pilot-scale level in which small-scale single cell protein production is reported.

**The Jordanian Centre of Veterinary Vaccines**

The centre is currently involved in the production of a number of veterinary vaccines of importance to Jordan at a capacity which ensures the full coverage of all local needs. In addition to vaccine production, a number of research activities are attempting to improve the production system and to produce new vaccines. (Source: Jordanian Royal Scientific Society report of August 1989)

**The Netherlands**

Biotechnology at TNO

In 1988 some 200 TNO scientists and co-workers were involved in about 150 biotechnological research projects with an aggregate budget of more than Dfl. 30 million. More than half of the budget concerned contract research.

TNO's biotechnology programme covers a wide range of application-oriented research activities. Besides R&D in the field of environmental technology, TNO carries out biotechnological research for the food, nutrition and cattle fodder industries, the pharmaceutical industry, the chemical industry and the apparatus and equipment industry.

Biotechnology at TNO is carried out in three divisions: Nutrition and Food Research, Technology for Society, and Health Research. The tasks are divided in such a way that research is performed by those laboratories that traditionally have the most expertise available in the area concerned.

An Expert Committee on Biotechnology, consisting of representatives of the above divisions, co-ordinates the execution of TNO's vast biotechnology programme. The Committee recently published a representative selection from this programme in the booklet "TNO biotechnology". The booklet is meant to illustrate the fields where collaboration with third parties is possible.

Research items have been grouped according to possible field of application, i.e. food and feed, pharmacy, chemistry, equipment and environmental technology. Each item starts with background information about the reasons for the research. Furthermore, details are provided about the TNO research in progress, possible applications, collaborations and contacts. As the techniques and experiences described are often also applicable to other fields, the booklet has been provided with an index of keywords.

For a free copy of "TNO biotechnology, a selection from present research" please contact: Mr. B.A. Heide, TNO Expert Committee on Biotechnology, P.O. Box 108, 3700 AC Zeist, Netherlands. (Source: Applied Research, June 1989 25)

**Norway**

Biotechnology in Norway

Research and development in biotechnology has been given high priority in Norway since 1985. In the three-years period 1986-88 about NOK 200 million was invested in research by four of the Norwegian research councils. The concerted effort to improve the scientific capability of the participating research institutions has been co-ordinated by a national committee on biotechnology. The committee has just presented a plan of action for the 1990-92 period. The focus of the plan will be on basic and applied research within the following seven areas: cell and gene technology, medicine, agriculture, aquaculture, industry, environment and biotechnology for the developing countries. Protein and polysaccharide engineering are included under cell and gene technology. To effectuate the ambitious plan will require an investment of nearly

NOK 500 million over the three year period. The research will be performed by Norway's universities, independent research institutions and industry.

For a small country like Norway it is important to concentrate research on areas of special significance. The strategy adopted involves the universities being responsible for all the important basic disciplines required for giving students of biotechnology first class training and high qualifications, but limiting applied research to certain preferred areas and with some degree of specialization between the various universities and research institutions. In this summary three areas of note will be covered briefly.

The aquaculture industry has grown very rapidly in Norway in recent years, and is now of considerable economic significance. The future development of aquaculture will depend on research aimed at solving problems in many areas. Biotechnology will be of central importance in areas like disease control (prevention, diagnosis, treatment, vaccine production) and feed production. Basic and applied research in all these areas are under way. The industry is dominated by a few species, salmon and trout currently being the main products. Several laboratories are developing methods for the large-scale cultivation of various marine species. The success of these research programmes will depend on a biotechnological approach in areas like developmental biology and the production of efficient feed for fish larvae.

In addition to the aquaculture industry there are several sectors where Norwegian industry may be able to compete in the international market. One such field is marine biotechnology and in particular the production of marine polysaccharides. Norwegian industry is a major producer of alginate and chitosan. The potential of these marine polysaccharides has been extended considerably in recent years. Research and development is focusing on obtaining better understanding of the structure/property relationships, the development of efficient methods for production and use of ultra pure qualities of alginate and chitosan, and on enzymatic and genetic modifications of the biosynthetic machinery leading to the production of biopolymers with modified properties.

Another area of considerable promise for Norwegian industry is the biotechnological applications of the highly monodisperse particles produced by Dyno Industries. These unique particles may be produced with varied composition, morphology and size (1-100 microns). They have a large industrial potential in separation technology (separation of cells and chromatography) and for several analytical applications (immunochemical methods). (Source: BIO Technica Journal, No. 2)

## Switzerland

### Biotechnology in Switzerland

**Biotechnological research**

Swiss extended infrastructure for the scientific support of biotechnology includes several university research centres for biotechnology: the Institute for Biotechnology of ETH Zurich, the Institute for Medicine and Chemistry of Bern University, the Bio Center of Basel University and the Institute for Immunology in Basel.

A major source of research funding is the Swiss National Fund. More than 38 per cent of its grants in 1987 were allocated to biological and medical research which means a total of 80.7 million Swiss francs. Priority is given to projects related to isolation, synthesis and bio-synthesis of natural substances.

Scientific research in biotechnology even led to a Nobel Prize in 1987 when the Swiss professor for microbiology Werner Arber at the Bio-Center of the University Basel was honoured for his discovery and application of restriction enzymes, together with the American professors Daniel Nathans and Hamilton O. Smith.

Politicians are critical that a co-ordinated national programme to support research in biotechnology does not exist in Switzerland. One reason is the extensive industrial research by the large chemical companies although an offensive strategy would be desirable to improve public research infrastructure and to preserve international competitivenes mainly for small and medium enterprises.

**Specialization of industry**

Within the large field of biotechnology applications Swiss companies have gained international leadership for biotechnological processes. Some examples may demonstrate this:

- Environment

The bacteriostatic effect of low amounts of silver is used to sterilize and preserve water. Ions of silver make micro-organisms in drinking water inactive. This allows the production of drinking water from delicate water sources.

- Agriculture

Intensive research work is done in Switzerland on the application of biotic processes in agriculture. For example, numerous plans are worked out for economic and environmentally acceptable fertilizing, at present mainly by nitrogen.

- Drugs and chemicals

In the sectors of drugs and industrial chemicals the large chemical groups at Basel are keeping several leading positions while even small companies are finding market niches like a small Zurich company producing natural substances and derivates by biotechnological processes.

- Equipment

In the equipment sector several Swiss firms are developing bioreactors for laboratory application including hardware and software for process control.

In general, it may be expected that the internatonally active big Swiss chemical companies in the medium-term will keep or extend their position in the biotechnology market.

Questions may arise about the consequences for small and medium enterprises. They are applying traditional chemical processes which may be partially replaced by biotechnological processes within the ten years to come. If they leave biotechnology aside, they may loose competitivity in several sectors.

On the other hand, entering biotechnology requires high investments and sufficient personnel and financial resources. To overcome the narrow

operational limits of small and medium-sized enterprises, university and private consulting teams are trying to lower the threshold for entering biotechnology and to arrange for praxis-oriented research co-operations.

**The environment**

Swiss enterprises active in biotechnology are also participating in special trade fairs. One of the fairs on laboratory and production technology (Ilmac) is organized by the Swiss Association of Chemists which is also engaged in conducting conferences on research, new methods and sectors in chemistry and associated sciences. The journal "Swiss Biotech" serves the communication needs of the Swiss biotechnology community.

To sum up, Switzerland holds strong positions in biotechnology. The commercial field is dominated by the large Swiss chemical companies. Small and medium-sized companies as well as newcomers cover the subcontracting market. They include equipment manufactures, consulting firms and producers of basic substances for research and laboratories. The most successful strategy for small biotechnology companies in Switzerland is to look for market niches and to offer specialities. (Source: BIO Technica Journal, No. 1)

**Spain**

Spanish biotechnology

Spain represents one of the last growth markets in Europe, according to Consulting Resources Corporation of Lexington. Mass. One of the key areas receiving the Government's attention is biotechnology. As one example of its increasing interest, the Spanish Government has initiated the National Mobilization Programme in Biotechnology, a multi-million dollar effort to fund and support R&D in this field. Recently, a new National Biotechnology Centre has been established under this programme.

Today, almost 50 Spanish companies report an active or planned involvement in biobusiness. Included in the list of active companies are: BioKit, Antibioticos, Laboratorios Alter, Ingenasa, Laboratories Menarini, Invesgen, and Processes Enzymaticos. Currently, their product development interests are mainly in diagnostics and pharmaceuticals, although the agri-food sector is likely to be affected in the future as well. (Source: Chemical Marketing Reporter, 24 April 1989)

**United Kingdom**

Royal Commission calls for tougher controls

Government proposals for controlling the release of genetically engineered organisms (GEOs) into the environment do not go far enough, according to a report* by the Royal Commission on Environmental Pollution. The Department of the Environment (DoE) said however, that it will take account of the Commission's report in drawing up legislation for a new "green Bill".

Both the DoE and the Health and Safety Commission (HSC) should approve the uncontained release of a GEO, the Commission concludes. Release without consent should be a criminal offence, in contrast to the present voluntary notification scheme operated by the HSC.

A "release committee" of experts in a wide variety of subjects, such as biology, genetic engineering but also ecology, should be set up, according to the report, in a departure from the government proposals. Applications for the release of GEOs should be considered case by case until categories can be established. The release committee should advise both the DoE and HSC.

The Commission is also "unhappy" that the government proposals restrict themselves to areas where no other regulations exist.

While the government proposals apparently only apply to trial releases, the Commission wants to extend controls to commercial products. Additionally, a register of "releasers" should be kept. The report also goes beyond the government proposals in recommending extensive monitoring of the releases as a condition to granting of a licence.

Concern about industry interests has tempered the report, according to the Commission. New regulations should not be cumbersome, and should be in the industry's own interest, it believes. Because of the strict liability that is to be applied to releases of genetically engineered products, tough regulation will be cheaper for the industry in the long term, the Commission says.

Close co-operation with international and European agencies is essential according to the report. GEOs ability to cross boundaries make national regulations almost meaningless if no international controls exist. The option of companies releasing GEOs in countries with the least regulations, should be avoided, the report says.

A working party to look into risk assessment procedures, called GENHAZ, has been established along the lines of HAZOP procedures used in the chemical industry. GENHAZ procedures are to be finalized by the end of the year. (Source: Chemistry and Industry, 17 July 1989)

Biotechnology Directorate

Acting promptly on the recommendation of a review panel, the Science and Engineering Research Council has prolonged the life of its Biotechnology Directorate for another six years and has formed a joint advisory board for biotechnology with the Department of Trade and Industry.

The new board will deal with matters of common interest to the council and the department, ranging from training and research to regulations and technology transfer, and will further strengthen the collaboration growing between the council's Biotechnology Directorate and the department's Biotechnology Unit.

Both parties say the board will help increase the transfer of research into the marketplace. (Extracted from Bio/Technology, Vol. 7, April 1989)

Lift for protein research

The British Government has approved two research programmes in biotechnology, both through its LINK scheme to support collaborative work

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* "The release of genetically engineered organisms to the environment", 13th report, HMSO, 13.90 pounds sterling.

between academia and industry. The first will funnel 9.6 million pounds sterling into protein engineering over the next five years, and has the support of the Ministry of Defence and three of the research councils. The second is worth 7.5 million pounds sterling over five years, and will support research on the industrial applications of biotechnology, including research into novel processes for extracting products, new designs for fermenters and ways of protecting the environment from the organisms produced during such processes.

Protein engineering is a relatively new field of research by which scientists aim to introduce deliberate and useful changes into the structure of proteins, and ultimately to design new proteins. The Medical Research Council hopes to finalize plans over the next few months for an Interdisciplinary Research Centre in protein engineering, to be based at Cambridge University. (Source: New Scientist, 1 July 1989)

### 7.5 million pounds sterling, 5-year LINK biochemical engineering programme launched

On 15 June, the Government launched a new 5-year, 7.5 million pounds sterling research programme which aims to increase industrial use of biotechnology. The Biochemical Engineering Programme, part of the Government's LINK initiative, will support collaboration between industry and the scientific community. Funding will be provided by the Department of Trade and Industry (4.3 million pounds sterling) and the Science and Engineering Research Council (3.2 million pounds sterling). Individual projects will be funded 50:50 by industry and Government.

Biochemical engineering is concerned with the development of equipment and processes to exploit biotechnology on an industrial scale. The programme will address four priority areas: (1) innovative downstream processing; (2) fermentation technology; (3) process control in biotechnology; (4) containment, asepsis, sterility and the environment - involving improved barrier technologies for the exclusion of external environment from sensitive bioprocesses and containment of potentially hazardous agents within bioprocesses. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

### Virus research unit set up

A unit is being set up in Oxford, UK, to carry ou pre-competitive research which could lead to the development of a whole new class of drugs for the treatment of viruses and certain cancers.

The UK drug major, Glaxo, British Bio-technology Ltd. (BBL), a small Oxford healthcare firm, the UK Science and Research Council and the Department of Trade and Industry are each investing some 175,000 pounds sterling ($285,000) over four years to fund a virus research unit at Oxford University's department of biochemistry.

The virus molecular biology group, to be headed by Drs. Susan and Alan Kingsman, will investigate how viruses cause disease in humans. This will help them discover therapies which inactivate viruses inside the human cell.

The group intends to study the human papiloma virus, implicated in cervical cancer, and the AIDS virus to learn more about the control or "switch" genes which enable it to multiply. Scientists will then be able to screen for drugs which turn off the genes and stop the virus replicating.

Such a therapy would have to be taken for a life time. Obviously this type of therapeutic is potentially more profitable than a one-off vaccine. The techniques could also apply to other viruses.

The research is part of the LINK programme but differs in that only two firms are involved. Glaxo is interested in the broad scope of the project while BBL wishes to concentrate on the AIDS virus. Both will have their own programmes running within the project. (Source: European Chemical News, 22 May 1989)

### MRC unit in protein function and design

The Medical Research Council (MRC) has established a new unit in Cambridge, under the honorary direction of Professor Alan Fersht, FRS, who holds the Herchel Chair of Organic Chemistry at Cambridge University. The Unit's research programme will continue and expand Professor Fersht's pioneering work in protein engineering which has been largely carried out with MRC support.

By combining the powerful new tools of molecular genetics with those of physical enzymology and of structure determination, including magnetic resonance spectroscopy, his team have already made important discoveries about the ways in which the functions of proteins, especially enzymes, are affected by changes in the amino acid sequence introduced by the techniques of protein engineering.

The Unit's programme will form a central component of the Interdisciplinary Research Centre (IRC) in Protein Engineering, for which the MRC has recently received additional funds through the Science Vote and which should be in operation by early 1990. It will be housed in the University Chemical Laboratories and will have eight scientific staff, together with support staff and attached fellows and research students. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

### AFRC gene transfer grants

New grants, totalling over 500,000 pounds sterling, have been made by the Agriculture and Food Research Council (AFRC) to scientists at Dundee, Edinburgh, Glasgow and St. Andrews universities. Dr. C. Neil and Professor D.E. Onions at the University of Glasgow receive support for investigating the use of viruses to transfer commercially beneficial genes between species. To date, gene transfer in animals has been achieved by injecting genes into egg cells. It is hoped that using virus carriers will be more efficient and more cost-effective during commercialization.

Gene transfer offers a new approach to improving livestock production, yield and disease resistance. The group at Glasgow will work on transgenic sheep and collaborate closely with researchers at Edinburgh who have already introduced medically useful genes into sheep. The blood clotting factor missing in haemophiliacs can be produced safely in the milk of transgenic sheep carrying the human gene.

A new linked research programme is being established between the Hannah Research Institute at Ayr and the University of Leeds. It will identify important molecular interactions that determine stability and shelf life in foods such as mayonnaise

and cream liqueurs that are emulsions of oil and water. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

New glycobiology institute

Searle, the Chicago-based pharmaceutical company which is part of the Monsanto group, has agreed to provide 2.9 million pounds sterling to help fund a new research centre at Oxford University. The new Glycobiology Institute will enable collaborative research to be undertaken, involving scientists from both the University and Searle. The research is likely to bring major benefits in identifying potential biopharmaceuticals and ensuring that they become viable drugs.

The new Institute is designed to develop and exploit the future commercial potential of work done by an Oxford University team led by Professor Raymond Dwek, professor of glycobiology. The work was initiated in 1983 with a grant from Monsanto.

**Defective glycoproteins**

The new glycobiology centre will be run by Professor Dwek and clinical research co-director Dr. Thomas Rademacher. The centre is part of the Department of Biochemistry and will eventually employ some 60 scientists. It will be housed in a five-storey building, located next to the University Parks. Oxford University is putting 800,000 pounds sterling towards the new unit and a further 1.3 million pounds sterling towards a botany department, which will occupy two floors of the new building.

Although scientists can create many useful proteins, they still cannot recreate all of nature's essential molecules. Many essential proteins do not exist alone, but are combined with side chains. Frequently, these are sugars, as in the case of glycoproteins. Glycoproteins help molecules to bind and interact within the body.

Searle's interest in Professor Dwek's research relates to the therapeutic applications emerging from the association of disease with unique sugar defects on glycoproteins. The team's current work focuses on defective immunoglobulins (IgG) and the pathogenesis of rheumatoid arthritis. Additional research is examining defective glycoproteins related to Crohn's disease, tuberculosis, AIDS and cancer.

One of the more promising future applications for glycobiology is in screening populations for disease and developing diagnostic tests. If a sensitive enough test could be developed it might identify the presence of a disease by showing the absence of a specific sugar in a patient's blood serum. (Extracted from Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

Bridging the gap

A group of scientists from Cranfield Institute of Technology in Bedford, England, has formed a company to act as matchmaker between researchers and investors in biotechnology. The group complains that the UK is the pioneer in many advances in this new industry, yet British industry is too ignorant of biotechnology to spend money to turn ideas into marketable products. Such products might include biosensors which use micro organisms as detectors, developments in drugs, and the use of bacteria to get rid of pollution and to guarantee the safety of food. The market for biosensors alone has grown from 40 million to 550 million pounds sterling in four years.

Cranfield Biotechnology will make the Institute's researchers available to industry for consultancy and development work, and put academics and companies in touch from around the world. (Source: New Scientist, 22 April 1989)

EEC chooses university to lead "in-body" microsensor consortium

The Biotechnology Centre at the Cranfield Institute of Technology in Bedfordshire has been chosen by the European Economic Commission to lead an eleven country consortium established to investigate and further research the use of chemical microsensors within the body (in vivo monitoring)*. Cranfield's Biotechnology Centre is recognized as a world leader in the development of sensor technology for biological applications. Dr. Tony Turner, Head of the Bioelectronics Division at Cranfield will lead the consortium and a total of 23 centres in 11 countries within the EEC and Switzerland will participate in the programme.

The objective of the 5-year programme of concerted action is to bring together the leading experts in the fields of medicine, biochemistry and microsensor technology to focus on further developing this rapidly expanding area of science.

The action was initiated by the Biomedical Engineering Committee for Concerted Action (BME COMAC), which falls under the auspices of the Directorate General 12. Following an "expert meeting" and a workshop earlier this year, at which 35 prominent Engineering Science experts from all EEC member countries met to discuss the interface between biology and sensors, it was concluded that a Concerted Action proposal should be pursued. This was proposed and subsequently approved in November of this year.

In the face of intense and well funded research effort in the USA and Japan, there has been an increasing willingness on the part of European scientists to collaborate in this vitally important field.

Initially focus will be on the analysis of clinical problems, identify suitable analytes and consider sites within the body for continuous monitoring.

The EEC has provided initial funding of over 400,000 pounds sterling for the co ordinating programme to get underway, and consortium members in other warder states have already received national government funding to promote further research and development work in this area.

Recent advances in sensor technology have brought the goal of reliable, continuous "in vivo" sensing within the realms of possibility. A successful co ordination of the highly skilled, but dispersed European activity in this field will

* The term "in vivo chemical sensor" is reserved for devices used within the body, as opposed to "ex vivo chemical sensors" which require the delivery of the substance for analysis to a sensor outside the body.

undoubtedly enhance the level of medical care available to the population of the European Economic Community. It will also create important commercial opportunities for European companies over the next decade. (Source: News Release, No. 26 88)

New biotechnology firm

Some heavyweight academics and industry-based scientists are behind a new UK biotechnology company called Immunology Ltd. It has been set up to concentrate on cell biology and genetic engineering in order to develop the next generation of therapeutic products based on immunology.

Immunology has been established in Cambridge by Dr. Alan Munro, previously head of immunology division at Cambridge University, and Dr. Stephen Bunting, a director of Abingworth Management. (Source: Manufacturing Chemist, April 1989)

**United States of America**

US finalizes controls on genetic engineering

The administrator of the US's Environmental Protection Agency (EPA) is expected to decide this month on the final form of draft regulations controlling the release into the environment of new micro-organisms, including those produced by genetic engineering.

In contrast to European countries, regulators in the US do not distinguish between genetically engineered micro-organisms and other types. The regulations will be implemented under existing laws, such as the Toxic Substances Control Act.

A cabinet level committee decided in the mid-1980s that existing legislation could provide the necessary regulatory framework to govern the newly emerging biotechnology industry. Since then, the EPA, the US Department of Agriculture and the Federal Drug Administration have been writing regulations to apply existing laws to the products of biotechnology, including genetically engineered micro-organisms. The agencies have also funded the National Research Council to examine environmental issues. The council is expected to publish a report in the autumn.

In February, the EPA made available for public comment some 6,000 pages of documents about the regulation of the new micro organisms, including draft regulations. During the next few weeks, the agency's new administrator, William Reilly, will decide whether the draft regulations need modification in the light of the responses.

The EPA's proposed regulations allow the agency to vet all new micro organisms or uses of micro organisms. As proposed, the regulations control micro organisms developed for commercial markets and exempt micro organisms for research. One of the controversial questions to be settled is how to define whether or not a new micro organism is being developed for commercial applications. The agency has already examined about 50 applications from a number of research institutes to run field trials of genetically engineered micro organisms. It has given permission for at least 10, including the first release later this month of a genetically engineered virus.

Until new regulations are in place, the agency can neither force companies to comply with its conditions for granting permission nor punish infringements.

The EPA is paying $240,000 to support scientists at the Boyce Thompson Institute in New York State working on field trials of a genetically engineered virus that will be released later this month. The trial is one step in the development of genetically engineered viruses that will be more effective at killing moths and butterflies, whose caterpillars attack crops such as cabbages and cotton. The work is along similar lines to that being done at the Institute of Virology at Oxford.

The researchers at Boyce Thompson are concentrating on one of a number of baculoviruses that live in colonies embedded in plant proteins. When they are caterpillars, the insects eat lumps of protein containing the viruses, and the viruses can kill them. Eventually, the scientists aim to insert a new gene to code for a toxin that will enable the virus to kill the caterpillar more rapidly. But first they need to alter the virus so that in its more virulent form it will not survive long in the environment.

To do this, the scientists have removed the gene that codes for a coat of protein that protects the virus. Viruses without the coat are unlikely to survive for long: the trial should discover exactly how long. (Source: New Scientist, 15 July 1989)

USDA steps up efforts in biological control

Marking 100 years of biological control of agricultural pests - starting in 1889 with Vedalia beetles from Australia checking a California citrus pest - the Department of Agriculture is stepping up its biocontrol efforts. The USDA has signed two agreements calling for joint biocontrol research for the first time at Soviet laboratories. Work starts this summer in Kishinev and Leningrad on more than two dozen insects and weed pests. This spring, three ARS scientists go to China under a new five-year pact that includes a joint laboratory in Beijing. An ARS researcher has filed for patent protection on an improved virus to kill gypsy moths. A pilot project will use a fungus to fight wilt in New Jersey eggplants. Plans are afoot for a Biological Control Service Institute to serve as an international clearinghouse. (Abstracted with permission from Chemical Engineering News, 3 April 1989. Copyright (1989) American Chemical Society)

US biotechnology is in better health than was expected

The state of biotechnology in the US, especially in new drug development, is strong, according to results of surveys conducted by the Pharmaceutical Manufacturers Association. The surveys are on new medicines under development using genetic engineering and biotechnology patents issued in the US during the past three years.

PMA's latest survey of "Biotechnology Medicines in Development" shows that 80 genetically engineered drugs and vaccines are in clinical tests or are at the Food and Drug Administration for review. In contrast, a recent study by the FDA revealed that the US has twice as many pharmaceuticals approved for marketing or in clinical testing as Japan.

In addition, PMA's latest survey of US biotechnology patents issued found that the US leads

Japan and Western Europe in genetically engineered health care patents. Of the 182 genetic engineering health care patents issued in 1988, 155 were US owners. Japan received 15 and Western Europe nine.

Cancer is the main focus of pharmaceutical biotechnology research. More than half - 45 - of the medicines in development are for cancer or cancer-related conditions.

Biotechnology is an increasingly important tool in fighting AIDS. Fourteen products, almost twice as many as were listed in PMA's first survey last July, are being tested for AIDS or HIV-related illnesses.

Nine medicines and one vaccine are at the FDA for review.

Ten medicines and one vaccine are in the final stage of clinical testing.

Three research projects have been completed and approved by the FDA. Two interferons, "Roferon"-A by Hoffmann-La Roche and "Intron" A by Schering-Plough, have been approved for treatment of AIDS-related Kaposi's sarcoma and "Hibtiter" by Praxis Biologics, a conjugate vaccine, has been approved for haemophilus influenza type B.

Forty-five companies are involved in developing the 80 medicines. PMA members and affiliates are involved in more than three-quarters of those research projects. Key findings of the US patent survey are:

- 1,391 biotechnology patents were issued by the US Office of Patents and Trademarks in 1988;
- 837 of them were pharmaceutical/health care patents;
- 237 of the patents issued use genetic engineering, more than double the number issued in 1986;
- Well over three-quarters (182) of the genetically engineered biotechnology patents last year were pharmaceutical/health care related. That is more than double the number issued in 1986.
- The US leads in the field of genetic engineering and in the application of advanced biotechnology techniques for the discovery and development of new medicines 195 of the 1988 genetically engineered patents (82 per cent) were of US origin and 155 of the genetically engineered pharmaceutical patents (85 per cent) were issued to US owners.
- US corporations were the largest single source of genetically engineered patents (108) and genetically engineered health care patents (84) in 1988. (Source: Chemical Marketing Reporter, 29 May 1989)

Field testing of carp approved

The biotechnology review board of the United States Department of Agriculture has approved the first field test involving a genetically engineered fish. Researchers at Auburn University in Alabama have been given permission to introduce carp which contain trout growth hormone genes into a test pond at the university.

The carp were developed through a collaboration between researchers at Auburn, and at Johns Hopkins University in Maryland. Carp containing the trout genes have been shown in the laboratory to grow significantly larger than normal carp, a finding that could have commercial significance for fish farming.

The carp will be kept in a contained pond, with a series of barriers to prevent their escape into open water. As an added precaution, a mechanism has been developed to introduce poison into the water should a fish manage to get beyond one of the barriers.

The Auburn carp is the first transgenic animal to be approved for release into the environment by the Agricultural Biotechnology Research Advisory Committee, which has been charged with developing guidelines for reviewing field-test applications from researchers working under Department of Agriculture grants. The finishing touches are now being put to the committee's guidelines, which should be published in the Federal Register for public comment within the next two months. (Source: Nature, Vol. 338, 30 March 1989)

Genome sequencing of the nematode

The question of which person's genes will be mapped and sequenced by the US human genome initiative is still open, but under the genome programme of the US National Institutes of Health (NIH), the humble nematode may be the first higher organism to have its genes ordered into a physical map. At its second meeting, NIH's advisory committee on the human genome project unanimously agreed to endorse a joint effort by US and UK researchers to finish the nematode gene map.

It has always been clear that the genome effort will not provide the sequence of one individual's genes, but rather an amalgam of human DNA from various sources. But mapping and sequencing human genes will be only a part of the genome initiative: further studies of the genomes of such well-studied "model organisms" as the Escherichia coli bacterium, the fruit fly Drosophila, the nematode and the mouse have been discussed as stepping-stones towards the human genome.

Researchers at the British Medical Research Council's (MRC) Laboratory of Molecular Biology at Cambridge and at Washington University Medical School in St. Louis, Missouri, have broken down the genome of the nematode into 200 sets of overlapping DNA fragments, or "contigs".

By bridging breaks between contigs using the yeast artificial chromosome technique developed at Washington University, James Watson, director of the NIH Office of Human Genome Activities, believes the team can whittle the nematode genome down to 100 contigs in roughly a year. Putting the contigs in their correct order will provide a physical map of the nematode, which he believes can then be sequenced by a team of 50 technicians within six years.

Watson estimates that a three year $600,000 grant would allow the US British collaboration to finish the physical map and start sequencing. At 100 million base pairs of DNA, the nematode genome is roughly the size of an average human chromosome. The nematode's biology is so well understood it is known to have exactly 958 cells, and each cell division during development has been completely

described. Watson says "the worm people may lead the way" in genome research. (Source: Nature, Vol. 339, 29 June 1989)

Human genome project costs daunting

Manufacturers developing technology for DNA sequencing need to find ways to cut the cost down to a penny base and increase the speed of the process if the project to sequence the whole of the human genome is to become a reality, Dr. Lloyd Smith told delegates to the Pittcom Analytical Science meeting in Atlanta, Georgia.

The size of the project in genetic information terms was daunting. The human genome is estimated to consist of 300,000 genes - some 3,000 million nucleotide bases.

Allowing for at least one repeat of sequencing at each base, at today's costs of around one dollar/base, the project would cost in the order of $6 billion, a figure which Dr. Smith said would be unacceptable to funding agencies.

The speed of current DNA sequencing technology also makes the viability of the much discussed project seem dubious, Smith suggested. The current systems, which uses fluorescent dyes to detect nucleotide bases can work accurately to a maximum of 300 bases/run, each run taking 14 hours.

The human genome project could take more than a thousand years at such speeds said Smith. Even with reduction of costs of sequencing each base, the project would be horrendously expensive.

There were several other fundamental problems which needed to be solved before the project could begin in earnest. DNA could be squeezed in manageable chunks of between 500 and 1,000 bases. But with individual human chromosomes consisting of 1,000 million bases and more, a means of fragmenting and purifying chromosomes into perhaps, 40 kilobase units needed to be developed. These would further need to be split for sequencing.

There was a further complication in that methods would be needed to sort out the order of the message, once it had been broken down into small sub-units. Methods currently used for cloning short DNA sequences would need to be automated, as would the processing of data.

Smith said he believed it would take more than ten years for current methodologies for sequencing to be enhanced to the level whereby the project could become financially acceptable. (Source: Manufacturing Chemist, April 1989)

Computer array interprets the human genome

Computer techniques from California will soon be making a rapid analysis of the billions of bits of information that make up the human genome, according to scientists at Washington D.C. Leroy Hood, a biologist at the California Institute of Technology in Pasadena, led the effort to design an array of computer chips for scanning and interpreting the chemical bases that make up the human genome.

He unveiled the basis of the technology, which is called the Fast Data Finder, at a meeting on the human genome. Two companies, TRW, a Californian electronics company, and Applied Biosystems, a Californian biotechnology company, designed and built the array of chips which make up the system.

Machines can determine sequences in DNA far more quickly than humans, and recently biologists have programmed computers to seek unusual sequences.

The chips deal with a stream of data, consisting of strings of four letters, which represent four nucleotides. These strings pass through up to 10,000 processors in series. The system identifies so-called motifs - patterns of genetic material known to have valuable functions. A transposed base in a sequence, for example, may indicate a predisposition to heart disease or muscular dystrophy.

The system can also compare newly discovered sequences with an existing database, to pick out strings that match. Such matches provide clues to the function of the sequence. Hood says that his system can search 27 million nucleotide bases in three seconds. This compares with a time varying from several hours to three-and-a-half days for current computerized systems. (Source: New Scientist, 6 May 1989)

NIH regulation on experiments abroad

Genetic engineering experiments funded by the US National Institutes of Health may not be done in a foreign country unless the host country has approved the experiments. The new NIH rules provide that the experiments must comply with the host country's laws on the subject or else must be reviewed by an NIH-approved board. The NIH review must then be accepted by the proper authority in the host country. In any case, NIH funded research must be in compliance with NIH guidelines that would apply in the US. (Extracted from Science News, 15 April 1989)

Biotechnology curbs opposed by traders

A new rule controlling export of genetically engineered micro-organisms will impose unwarranted burdens on industry and Government, according to a biotechnology trade group. The Industrial Biotechnology Association recommended "refined wording" in a response to a February Federal Register request for comments on the scope of biological export controls.

The Association says its wording maintains the integrity of the rule by ensuring reasonable export restrictions to regulate the movement of biological micro-organisms of legitimate concern without impeding the flow of scientific research.

According to the new, sweeping Department of Commerce rule, certain chemicals and biological agents exported to all countries, except Canada, are subject to licensing requirements, in response to national security concerns about international shipment of biological weapons. (Source: Chemical Marketing Reporter, 24 April 1989)

Biomedical bugs

The bargain sale of US financed research data to Japan was not the only conflict of interest to exercise the Human Resources and Intergovernmental Relations subcommittee hearing.

Biomedical researchers were seen as particularly likely to suffer conflicting interests, given the huge sums of money from pharmaceutical companies that supplement federal funds for drug development and clinical trials.

Commercialization "has been more aggressive, more brazen, and more experimental than other disciplines", according to Sheldon Krimsky, a

professor of urban and environmental policy at Tufts University. He claimed that there is evidence that refusal to share data and biological materials is increasing, even among scientists working for the federal government, implying that "public funds are being used to support proprietary knowledge".

Krimsky believes that new relations between universities and industry, such as Boston University's controversial $25 million investment in the Seragen biotechnology company, provide a "recipe for conflict of interest". According to his own survey, 45 per cent of Harvard University's biomedical faculty now have formal affiliations with 36 different biotechnology companies. (Source: Nature, Vol. 339, 22 June 1989)

FDA opens up drug access for most-serious AIDS patients

Continuing to step up efforts to give AIDS patients quicker access to drugs, the Food and Drug Administration has taken action on treatments for two of the most serious complications of the disease.

FDA commissioner Frank Young says the agency has approved the drug ganciclovir to treat an eye infection, cytomegalovirus (CMV) retinitis, that can sometimes lead to blindness in AIDS and other immune supressed patients.

In addition, Dr. Young says the Government is permitting expanded premarket distribution of an experimental protein product to treat the severe anaemia that weakens nearly half the 20,000 patients currently taking AZT (zidovudine) for AIDS.

Recent studies of about 100 AIDS patients with AZT-linked anaemia showed a protein product, r-erythrepoietin or EPO, reduced or eliminated the need for transfusions.

The FDA has also approved another EPO product for use in kidney patients with severe anaemia, but the genetically engineered drug is not officially sanctioned for use in AIDS patients and is undergoing tests for that purpose. (Extracted from Chemical Marketing Reporter, 3 July 1989)

## C. RESEARCH

### Research on human genes

Two genes may help regulate cancer cells

Key genetic regulatory processes might be controlled with new substances to treat cancer, according to K. Cowan of the US National Institutes of Health. Two genes have now been located that help regulate cancer cells: one codes for glutathione S transferase (GST Pi), which is used to help neutralize poisons even in normal cells; a second gene produces a protein that helps pump toxins out of cells, sometimes producing multi drug resistance (MDR). The genes might be located with the help of gene probes. NCI's P. Steeg has developed a gene probe to locate the NM23 gene that is present in benign breast tumours but absent from malignant ones. NM23 might therefore be a suppressor gene that keeps cancer cells from multiplying.

Gene probes to detect oncogenes could be useful in the diagnosis of cancers such as chronic myelogenous leukaemia (CML). Oncogene Science has received the US Federal Drug Administration's permission to market a gene probe that can detect leukaemic cells, thus diagnosing the disease. Other cancers are being increasingly associated with genetic changes, so that gene probes could be useful diagnostic tools. So far, gene probes are commercially available only for the rarer cancers but work is progressing on developing probes for the more common ones, with the aim of guiding therapy. (Extracted from New Scientist, 18 March 1989)

IL-2 and diphtheria toxin used against T-cell leukaemia

A linkage of IL-2 and diphtheria toxin may selectively kill cancerous T-cells, according to J. R. Murphy of University Hospital (Boston) and T. Strom of Beth Israel Hospital. Clinical trials have started. Animal trials in mice and monkeys show the "fusion protein" to be a hundred times as effective as ordinary diphtheria toxin without being too toxic to the animals themselves. There will be some toxicity to non-malignant T-cells, but this is not considered too risky. Patients with adult T-cells leukaemia and IL-2 receptor positive lymphomas will be treated with the new drug. Such patients would normally be dead five months after diagnosis. (Extracted from Medical World, 27 March 1989)

Search narrows for melanoma gene

Studies of skin cancer in six families have helped a team of scientists to focus on a gene believed to cause malignant melanoma, one of the most virulent types of cancer and one of the few showing an increased incidence in developed countries. The scientists now know that the gene sits on one arm of chromosome 1.

The scientists traced the gene to a section of the chromosome's short arm. They still seek the gene itself, but have discovered a molecular marker that is inherited with the gene. They now can start searching for the gene itself among the 13 million base pairs, the smallest units of genes, where they know the gene resides.

Sherri Bale of the National Cancer Institute, Nicholas Dracopoli of the MIT, and scientists from the Collaborative Research Company and the University of Pennsylvania worked together on the studies. They examined DNA from 99 relatives and 26 spouses in the six families with histories of melanoma.

Thirty four family members had malignant melanoma, of whom 31 had both melanoma and dysplastic naevi. These are pigmented moles that foreshadow the onset of melanoma.

The incidence of malignant melanoma is rising faster than any other form of cancer in the US, with about 27,000 new cases every year, a growth rate of 7 per cent. The genetic link was made with people with the inherited form of the disease, which accounts for only about 10 per cent of cases.

Six years ago scientists found that melanoma was sometimes inherited in families along with the rhesus blood grouping, which was known to be governed by a gene on chromosome 1. The researchers applied DNA probes to the DNA of family members with dysplastic naevi and melanoma.

The scientists also found that the trait for dysplastic naevi and melanoma is dominant: if one parent has the gene, a child has a 50 per-cent

chance of inheriting it, rather than a 25 per-cent chance as in many genetic diseases. The chances of getting the disease if the gene is present exceed 90 per cent, says Sale.

It could take several genes to cause melanoma. As for melanoma from sunlight, whatever transforms normal skin cells into cancerous ones may also trigger the gene this team is close to locating. (Source: New Scientist, 3 June 1989)

DNA-protein crosslinks

The changes that occur when a foreign substance binds to DNA have been identified, according to M. Dizdaoglu of the US National Institute of Standards and Technology. Such adducts, referred to as DNA-protein crosslinks, are thought to be critical in the generation of many cancers. DNA normally wraps around proteins, but changes in the proteins can be induced with gamma radiation, which produces free radicals that easily bind with other available biological molecules. It may be that similar adducts are created by free radicals in cells. DNA probes might now be developed to search for specific adducts. (Extracted from Science News, 1 April 1989)

Single gene may predispose people to allergy

Researchers at the Churchill Hospital in Oxford, England, have located a single gene that predisposes people to allergy. It is a dominant gene, which means that the "healthy" version of the same gene cannot mask its effects.

The idea of allergy as an inherited disease is not new. Every doctor knows of "atopic families" where asthma, hay fever and other allergic problems recur in each generation. But previous genetic studies have failed to identify the pattern of inheritance.

The new study has succeeded by using more flexible and sensitive criteria to identify allergic individuals. Some earlier studies looked for allergic symptoms, others tested the blood for high levels of IgE (the antibody that plays a key role in allergy). This new study offers three possible ways in which an individual can qualify as allergy-prone: high levels of total IgE in the blood, high levels of IgE to specific antigens (such as pollen), or a positive skin-prick test to one or more common antigens. The skin-prick test, which introduces minute amounts of antigen into the skin, is a standard diagnostic test in allergy.

The researchers used these flexible criteria to classify the members of seven large atopic families. They then analysed their genetic make up using probes that are specific for highly variable regions of DNA. The results from the first 16 probes were disappointing, but the seventeenth probe showed linkage with an "allergy gene", indicating that they are close together on the same chromosome. The probe is specific for the long arm of chromosome 11.

Chromosome 11 carries many genes for cell-surface markers, which play a vital role in communication between different immune cells. Defects in communication could lead to faulty suppression of IgE production. The researchers hope eventually to pinpoint the gene and identify its product, with a view to devising a drug that can block its effects.

More immediately, the test could be useful in identifying newborn babies carrying the gene. Although the gene predisposes individuals to allergy, actual disease may be avoidable - some of those classified as "allergic" in this study had no actual symptoms. While genetic background probably plays a part here, environment is also a factor. Earlier studies have shown that atopic families can reduce the likelihood of allergies in their children by avoiding risk factors in the first few years of life. (Source: New Scientist, 24 June 1989)

Progress on sickle cell anaemia

Sickle cell anaemia is well understood on a cellular and molecular level, according to the Lawrence Berkeley Laboratory (Berkeley, CA). However, there has never been an animal model for the disease. "Without an animal model, proposed treatments cannot be tested, and the pathophysiology of a disease cannot be followed", says Edward Rubin, a human geneticist in the Cell and Molecular Biology Division. Now Rubin has genetically engineered a human sickle cell gene into mice. But since the transgenic mice express only one of the human haemoglobin chains necessary to the polymerization that sickles red blood cells, the characteristic deformation does not occur. Next Rubin hopes to develop mice that produce both types of human haemoglobin chains or a human haemoglobin that polymerizes more readily. (Source: Chemical Week, 17 May 1989)

Mitochondria unravel the secrets of growing old

We grow old and infirm because of build-up of mutations in our mitochondria - the bodies in cells that produce energy. This is the finding of researchers in Australia and Japan, who claim that it should be possible to compensate for the damage caused by the faulty genes.

Anthony Linnane and his colleague at Monash University in Victoria, working with Takayuki Ozawa and his colleague at the University of Nagoya, say that DNA carried in the mitochondria mutates much faster than the chromosomal DNA of the nucleus. Over the years, they argue, the mistakes in the genes of the mitochondria accumulate and impair the ability of the organelles to produce energy efficiently through metabolizing oxygen.

Experimental evidence in support of this theory comes from Ian Trounce and his colleagues at the University of Melbourne and St. Vincent's Hospital in Fitzroy, Victoria. They analysed samples of muscle from 29 people aged between 16 and 92, and found a marked decline with age in the rate at which mitochondria in the muscle could generate energy using oxygen. The muscles of people aged over 75 generated energy at half the rate of those under 40.

The researchers speculate that their findings could help to explain why, for instance, the speed at which people run marathons falls with age. But a deterioration in the functioning of mitochondria could have much more widespread effects, because they are the major source of energy in most organs, including the brain. A decline in the efficiency with which cells generate energy from oxygen could, they say, have "a role in senescence in general". Drugs that circumvent particular complexes of defective enzymes in the mitochondria might help to prevent the decline, say Linnane and his colleagues.

Respiration at the cellular level involves the transfer of electrons along a chain of molecules. So-called "redox" substances, which lose and gain electrons easily, might help this transfer. Ascorbic acid (vitamin C) is one such redox substance. Others are menadione (a form of vitamin K) and ubiquinol (an enzyme).

"If our hypothesis is validated, new compounds with specific redox potentials, designed to react with the different electron transport complexes, can be rationally developed", the researchers conclude. (Source: New Scientist, 6 May 1989)

**Research on animal genes**

Genetic engineering in fish farming

If fish farmers cannot breed the perfect fish, perhaps genetic engineers could do better by direct manipulation of a fish's genetic material. Fish are difficult subjects for breeders, but they have many advantages for the genetic engineer. Unlike the eggs of mammals, fish eggs are easy to handle and to inject with the tiny glass needles that are used to transfer genetic material. The eggs are large; there are lots of them and they grow easily in culture. It is surprising, then, that genetic engineers have achieved so little.

Scientists are just beginning to overcome some of the early difficulties. One of the most fundamental problems of genetic manipulation of fish is that their eggs have a very small nucleus. The scientist must inject genes into the cytoplasm of the egg rather than into the nucleus, which may then fail to take up the genes.

In some experiments, offspring of injected fish had none of the injected gene, showing that it was not incorporated. In some cases, the injected genes are incorporated into the genome of only one of the first few cells of the fish. As a result, they are expressed in some, but not all of the fish's tissues. The fish is called a chimera. The gene may not reach the particular tissue where it must exert its effect. If it is not the fish's egg or sperm, the gene will not be passed to offspring.

After many attempts with different preparations of genes and injection techniques, however, scientists have successfully added genes to trout, salmon, catfish, carp and other farmed species. No implanted gene has had any observable effect on the fish so far. Scientists at the Chinese Academy of Sciences report that when they insert the genes for growth hormone into carp, the carp grow bigger. In a similar experiment at Auburn University in Alabama, Rex Dunham inserted genes for growth hormone from trout into carp. He reports that the engineered carp grew 20 per cent faster than normal. In both experiments, the bigger fish were within the normal range of sizes, so it was not clear if the gene had had a real effect. The genes may not have much effect because they are inserted with promoters (the sequence of DNA attached to an injected gene that causes it to be turned on and expressed) from rats or mice. The search is now on for fish promoters.

Genetic engineering raises the spectre of unnatural "altered" species. Perhaps optimistically, scientists expect fewer legal and ethical objections from the public to the manipulation of fish than they have faced with mammals. Some of the objections concern the release of altered organisms to the environment. Most countries have strict regulations governing experiments out of doors. But some fish are certain to escape from fish farms. Once loose, they can pass their added genes on to their wild relatives through interbreeding - with unpredictable results.

Some fish can be engineered so that this does not happen. Trout can be made triploid, containing three instead of two copies of their genes, with heat shocks or chemical treatments to eggs during their first division. Triploid trout are sterile. The technique is also possible with grass carp, but the eggs of other farmed fish have proved resistant to efforts to induce triploidy.

The main problem now facing prospective fish engineers is to identify the genes that fish farmers want in their stock. One overall characteristic they want is increased size, which may mean increased growth rate, or more efficient use of food. Growth hormone promotes both. Extra genes for growth hormone may not mean simple increases in growth, however. The extra hormone could have unpredictable effects on the growth and eating quality of fish. Scientists are toying with ideas for several genetic improvements in fish that might be more straightforward. One involves metallkothionein proteins, which bind heavy metals such as cadmium and mercury. These metals are increasingly polluting both fresh and sea water. A fish with a greater ability to bind heavy metals and neutralize them would tolerate higher levels of pollution.

Another target is the gene for "antifreeze" proteins, such as those made by winter flounder. Like antifreeze in cars, these proteins lower the freezing point of blood. Such a protein could prevent Atlantic salmon from freezing to death, as they often do in cages on farms. Scientists have identified the antifreeze gene but so far have tested it only in fruit flies.

Much of the work of developing libraries of gene sequences for other experimental animals must be repeated for fish before genetic manipulation will be profitable. Some biologists argue that it would be better to use classical techniques of breeding to solve problems such as resistance to disease before plunging into biotechnology.

Dunham has now reached the stage when he wants to test his fish outside the laboratory. He has submitted an environmental impact assessment to the US Department of Agriculture, asking for permission to test fish with introduced genes for human and fish growth hormones, in small outdoor ponds. The fish will be isolated by screens on the inlet and outlets for water, and by fences and bird netting. American scientists think the chances of gaining permission are promising. (Source: New Scientist, 22 April 1989)

**Research on plant genes**

Plant reactor to grow immobilized plant cells

Scientists in Shintaro Furusaki's group at Tokyo University have designed a bioreactor for growing immobilized plant cells - in this case, poppy cells that synthesize codeine. The poppy cells, which are immobilized in a matrix of calcium alginate, convert codeinone to codeine, a compound that is impractical to synthesize de novo. The immobilized poppy tissue calli synthesize codeine for five days: subsequent addition of the precursor codeinone resulted in only very low product yields.

In contrast, suspension cultures of the same cells maintain their synthetic capability after five days. Furusaki hypothesizes that loss of plant cell function may be caused by the restricted movement of nutrients and waste products in immobilized cultures. In fact, the researchers found that if they decreased the size of the tissue clumps, the cells lived longer and were nearly as efficient in synthesizing codeine as suspension cultures. (Source: Bio/Technology, Vol. 7, May 1989)

New light on evolution of flowering plants

Flowering plants may have existed on Earth some 200 million years earlier than can be confirmed by fossil evidence, suggest William Martin, Alfons Fieri, and Heinz Saedler of Max Planck Institute for Plant Breeding in Cologne, Federal Republic of Germany. Using changes in the nucleotide sequence of a slowly evolving enzyme called glyceraldehyde-3-phosphate dehydrogenase as a "molecular clock", the researchers calculate that the ancestors of modern flowering plants were already diversifying more than 300 million years ago. Fossil records for these plants go back only 120 million years. To confirm the accuracy of their clock, the researchers compared the nucleotide sequences of nine flowering plants, six animals and one yeast. They find an essentially uniform rate of change in the nucleotides both over this wide range of species and over time. (Reprinted with permission from Chemical Engineering News, 8 May 1989. Copyright 1989 American Chemical Society)

A quick way for hybrids to run to seed

British and Canadian scientists have devised a technique that may prove to be useful for plant breeders who are trying to improve crops. The technique was first tried by a team at the University of Cambridge in Britain two years ago, which sees it as a way of improving strains of wheat. But recent results from the Laval University in Canada suggest that the technique may also prove to be a tool for producing new plant hybrids.

Their approach uses ovules, the plant's egg cells, which have only one chromosome out of each of the pairs found in ordinary cells. The biotechnologists induce these ovules to form a seedling, by activating the ovule by some form of mock fertilization. If, during one round of division, the cells of the seedling are kept from splitting by the drug colchicine, but the chromosomes are allowed to replicate as usual, the resulting cells will each have the two identical copies of the ovule's genes that they need to form seeds.

If breeders treat the ovules from a hybrid plant in this way, they will obtain offspring from the hybrid that are much more genetically uniform than offspring obtained by the usual process of fertilization.

The technical problem is how to induce ovules to form seedlings. Scientists at Cambridge University discovered that if they place pollen from maize on the stigmas of wheat it will fertilize the ovules normally. The growing embryo jettisons the maize genes, leaving only the ovule's wheat genes. The researchers then treat the seedling with colchicine to obtain a mature plant, capable of producing seeds.

André Comeau and his colleagues at the Laval University have found that some of the maize genes in a few seedlings obtained this way stay in the plant until they sprout their first leaves. David Laurie, of the Plant Science Research Institute at Cambridge, says none of his team's hybrids has ever retained any maize genes.

The possibility that some strains of wheat may not jettison maize genes as quickly as others could mean that the wheat genes may be invaded by "transposons", or "jumping genes", lengths of DNA found in maize which move around among the chromosomes. This would spoil the genetic uniformity of the wheat.

However, this raises other possible applications for the technique, in mapping wheat genes. Transposons from maize can be traced within the hybrid plant's DNA. If a transposon jumps into a wheat gene and disrupts it, that cell might show a change in its behaviour. Such a change could prove to be desirable. Scientists can detect the transposon within the cell, and locate the gene that was disrupted and caused the change in the plant. (Source: New Scientist, 6 May 1989)

Transgenic plants prove a boon to basic research

The ability to produce transgenic plants has already resulted in a plethora of applications in fundamental research. We can now easily isolate and mass-produce in bacteria any plant gene whose product we know. Many important genes, however, are known only by their outward effects on an organism. One method that molecular biologists use to clone such genes is called transposon tagging. Transposons are elements of DNA that can move from one place on a chromosome to another. When such a transposable element moves inside a gene, the gene is inactivated, creating a mutant. We can identify the mutated gene by using the transposon as a DNA probe, to isolate the element and flanking DNA, and then use the flanking DNA as a probe to isolate the gene from a normal, "wild-type" plant.

Until recently, this approach was limited to those few species of plant that contain well-studied transposable elements, such as maize and snapdragons. But recently, J. Schell and his colleagues at the Max Planck Institute in Cologne have inserted a transposable element from maize into the genome of tobacco by using a Ti plasmid as a vector. They then devised a method for selecting plants in which the transposable element had moved. Researchers have since applied the technique to tomatoes, and it should work in a range of dicots.

Alternatively, the Ti plasmid itself can be used to cause mutations and tag genes of interest. Scientists at Monsanto have constructed a vector that allows biologists to select transformed cells in which the T DNA was inserted within a gene. Such selection is essential because higher plants have a large amount of repetitive, non-coding DNA into which T-DNA could insert itself.

Transgenic plants can also help researchers to study how genes work. Genes are controlled in part by neighbouring sequences of DNA known as promoters. A novel way of producing mutants for study is to produce plants in which the orientation of a gene is reversed with respect to its promoter. This results in an RNA with a sequence complementary to that of the normal messenger RNA - so called antisense RNA. This antisense RNA prevents the gene from making its normal product. By using antisense RNA to suppress synthesis of a key enzyme in flavonoid biosynthesis, a group in the Netherlands has produced transgenic petunia and tobacco plants with abnormal pigmentation.

In Australia, Jim Haseloff and Wayne Gerlach have developed another approach. They have exploited the natural ability of small strands of RNA, known as satellite RNA, to infect plants in conjunction with a "helper" virus. Replicating forms of these satellite RNAs can cut their own RNA at particular sites. The researchers have used this ability to design RNA enzymes, known as ribozymes. Ribozymes can be designed to cut a specific sequence of bases in an RNA molecule and offer a powerful alternative to antisense RNA in shutting off gene expression and creating mutants in plants. Researchers can link the ribozyme genes to promoters active only at temperatures higher than normal, to create temperature-sensitive mutants. This approach allows biologists to probe the functions of essential, as well as non-essential, genes.

Transgenic plants can tell us how viral genes work as well. For example, Steven Howell's group at the University of California at San Diego showed that plants carrying gene VI of cauliflower mosaic virus develop symptoms even when they are not infected by the whole virus, implicating that particular gene in the induction of symptoms.

Not all genes are active all the time, in all tissues. Many plant genes are regulated by stimuli such as light or hormones. Yet we still know little about how such control comes about. It can involve control at the level of messenger RNA, which in turn depends on interactions of proteins with DNA sequences some distance "upstream" from the gene.

Researchers have identified some of the sequences important in the control of gene expression by splicing one such upstream sequence to a "reporter" gene, which makes an enzyme that is easy to measure. For instance, we know that the small subunit (ss) of a major enzyme of the chloroplast, RBPC, is synthesized only in the presence of light. Leaves make the most, petals and seeds less, and roots hardly any molecules of this enzyme. By linking a segment of DNA upstream of this gene to a reporter gene and making transgenic tobacco plants, researchers have shown that the enzyme produced from the reporter gene is regulated in the same way as ssRBPC. They also demonstrated that the control of the gene involves sequences that act as an "enhancer" in leaves exposed to light, and a "silencer" to shut off expression in roots. Plant scientists are now using this method to study genes that control the ways in which plants grow and reproduce. (Source: New Scientist, 3 June 1989)

Plant ion-pump gene cloned

Scientists for the first time have cloned and sequenced a gene for an energy-generating protein that controls a higher plant's ability to take up nutrients from soil. The research might someday allow plant breeders to genetically engineer crops that more efficiently extract essential minerals through their roots, says molecular biologist and study leader Michael R. Sussman at the University of Wisconsin-Madison.

Better nutrient-extracting crops could live in deficient soils and so need little or no added fertilizer, reducing a farmer's costs and decreasing pollution from fertilizer run off.

The gene codes for a protein that crosses the outer membrane of al. plant and fungal cells and transports hydrogen ions from one side of the membrane to the other. The pump creates an electrical difference between a cell's inside and outside, producing "the most electricity of any protein in nature", Sussman says. A root cell uses this electricity to bring in soil nutrients.

Sussman and co-workers Jeffrey F. Harper and Terry K. Surowy wanted to study the gene for the pump from a plant of the mustard family, Arabidopsis thaliana, because of this plant's short lifespan and small number of genes. They used what they knew about the protein structure of an oat plant's pump to create a short genetic probe that pinpointed a partial-length oat gene. They then used this oat gene to find an equivalent full-length gene in A. thaliana.

Although researchers identified the pump gene in fungal DNA several years ago, it has proved more difficult to pick out in higher plants because the protein exists in tiny amounts.

Sussman and his research team are now trying to insert the newly identified gene into tobacco plants in a way that will cause the plants to produce an abnormally large number of proton pumps. (Source: Science News, 4 March 1989)

Genes as eco-safeguard

As researchers continue to develop genetically engineered products for applications in open environments, ecologists continue to voice concern over the potential effects. One attractive-sounding safeguard for large-scale uses entails the development of "suicide" or "conditionally lethal" mutations that, on signal, will lead engineered organisms to self-destruct.

The notion that conditionally lethal mutations may provide protection when dealing with micro-organisms harks back to early laboratory experiments involving crippled strains of genetically engineered Escherichia coli. Perhaps the key point to remember in evaluating the applicability of similar strategies for controlling environmental releases is that "complete containment cannot be achieved", says Ronald Atlas of the University of Louisville (Louisville, KY). Moreover, micro-organisms are being engineered to survive rather than fail in particular open environments, making the use of crippled laboratory strains out of the question.

Hence, using suicide genes appears attractive. The idea is that a micro-organism can carry an inducible gene that, once triggered, will kill the organism and thereby halt its spread in the open environment. The fundamental problem for this seemingly attractive approach is that "the scientific basis for it is not really there", Atlas says.

Despite limited research so far, there are hundreds - perhaps thousands - of genes of potential use in suicide schemes, says Stephen Cuskey of the Environmental Protection Agency (EPA, Gulf Breeze, FL). Care must be exercised in choosing them and the regulatory sequences used to control them. He recommends designing and testing a series of suicide "cassettes" that could be slipped into cells as needed. "There is a need for more than one cassette for redundancy, to ensure against loss of control by mutations", he says.

Atlas and his collaborators have been testing a lethal gene product encoded by E. coli and known as Hok (for host killer), a 55-amino-acid-containing peptide that kills the host and many other bacteria. They recently put the hok gene into an inducible plasmid, thereby creating a "suicide

vector". When induced, cells carrying this vector commit suicide - more or less. After a lag, cells appear that no longer carry the hok containing plasmid, Atlas says. However, when an antibiotic resistance gene was added to the suicide vector plasmid, "a mutation occurred and the suicide gene failed", Atlas says.

Besides straight suicide, other strategies for controlling agriculturally useful microbes are being contemplated. For instance, David Sands and his colleagues at Montana State University (Bozeman) are studying a native fungus, Sclerotinia sclerotiorum, as a means for controlling Canada thistle weed. Because the fungus also can kill several crop plants, Sands is seeking ways of limiting its host range through mutagenesis.

One such fungal mutant, which does not make cytosine, cannot kill plants in greenhouse tests, making it like "a Doberman on a leash", Sands says. If cytosine is sprayed on the plants along with the fungus, however, it again kills them. Another fungal mutant cannot form spores, and thus does not survive through winter. Comparable strategies may be applied to other engineered organisms.

Although suicide mutations provide some attractive schemes for helping protect the environment, some scientists do not believe they will be adequate to the task.

Nonetheless, biological containment has a lot of attractive elements not only for scientists but also for regulatory agencies. (Extracted from Bio/Technology, Vol. 7, May 1989)

**Research on yeast and fungus genes**

Trials success for "PCB eating" fungi

Researchers at Melbourne's La Trobe University are preparing to market a commercially viable and harmless process which can destroy organochlorines, such as PCBs, DDT, lindane and aldrin.

The chemicals are chlorine-containing aromatic compounds, with a molecular structure containing ring-like components similar to the benzene molecule. Current methods for permanently disposing of these chemicals require incineration at temperatures around 1,200°C for about 10 seconds. Inadequate heating causes only partial breakdown of the organochlorines, yielding intermediate products which can be as potentially harmful as the original products.

The Australian research team had already conducted unsuccessful trials with bacteria, so turned to fungi, particularly those responsible for causing white rot in timber. Their reasoning was based on earlier research which showed these fungi contained a complex enzyme capable of breaking down lignin.

Unlike most other enzyme systems which are highly specific to the chemical reaction they catalyse, the lignin-degrading system is less choosey. Thus, almost accidentally since chlorine containing organic compounds are very rare in nature, the white rot fungus can also digest PCBs, plus a range of other chemicals including lindane, DDT and possible dieldrin.

White rot fungi occur naturally worldwide and most grow only on woody plant residues. Few are parasites on living plants and even fewer on animals.

Another benefit is that the enzyme complex is not hindered by the relative insolubility in water of organochlorines, which tend to become absorbed on particulate matter, making them relatively inaccessible to enzymes requiring a soluble substrate - the fungal enzyme works outside the fungal cell on a natural substrate, lignin, that is itself insoluble in water. Additionally the organochlorines tend to bind to particulates rich in lignin.

In collaboration with the US consulting firm EnviroSearch and an American power utility, Professor Waid, who is leading the research, has begun large-scale field trials on a PCB contaminated site in the US. Preliminary results are said to be promising. The University is presently collaborating with a local company to promote and market the method internationally. (Source: Manufacturing Chemist, May 1989)

**Research on viral genes**

Synthetic molecule disables the 'flu virus

An Australian research team has announced that it had synthesized a simple molecule that disrupts the replication of the influenza virus in laboratory mice, reducing the severity of infection. The development, based on more than a decade of research into the structure of the influenza virus, represents a significant step towards a treatment for 'flu in humans.

Glaxo, the British pharmaceuticals company, signed an agreement with a small Australian company, Biota Holdings, which hold commercial rights to anti-influenza compounds emerging from the research project. Peter Colman, of the division of protein chemistry in Melbourne of the Australian national research organization, CSIRO, heads the project.

In 1983, Colman, an X-ray crystallographer, his colleague José Varghese at the CSIRO and Graeme Laver of the Australian National University in Canberra, described an unusual feature in the molecular structure of a protein on the surface of the influenza virus. The structure, a pocket-like cavity on the protein neuraminidase, appears to remain constant as the surrounding molecular landscape of the protein changes from one virus strain to another.

In the 1980s, scientists began searching for the chemical compounds that might interact with the virus's variable surface proteins and so stop it replicating. Researchers identified several compounds that showed promising anti-neuraminidase activity in vitro, but the compounds had no beneficial effect when they were tested in animals.

Colman and his colleagues persisted, and in 1986, the group at the CSIRO established a collaborative project with the nearby Victorian College of Pharmacy to synthesize simple molecules that would fit into the molecular pocket of neuraminidase. Mark von Itzstein led the project. The first molecule, tested in laboratory mice last December, showed slight but significant action against the virus. In each variant tested since, the antiviral activity has been more pronounced.

Colman says that such a compound will not actually cure people with 'flu, but it will suppress replication of the virus while the natural immune response deals with the reduced infection. 'Flu

victims may experience only a mild fever for a day or two. If and when a therapy for 'flu becomes available for people, it may have a dual action, helping to ward off the illness in uninfected individuals, and suppressing the virus in people who have already become infected. (Extracted from New Scientist, 24 June 1989)

Enzyme made to treat metabolic disease

Development of a treatment for Fabry's disease, a rare inborn error of metabolism, may be accelerated as a result of biotechnological production of α-galactosidase A by biochemistry professors David Calhoun and George Coppola of City College of the City University of New York. The disease, which afflicts 2,500 Americans, results from inability to break down a trihexosylceramide, which then accumulates in tissues. Symptoms include cataracts, pain and fever; death can come from heart or kidney failure. The researchers inserted the human α-galactosidase A gene into Autographa californica nuclear polyhedrosis virus, with which they infected cell cultures of the tall armyworm. This method eliminated purification difficulties and possibilities of viral contamination encountered in human cell cultures. Tuscon-based Research Corporation Technologies is licensing the patent and estimates the US market for the enzyme at $12 million per year. (Reprinted with permission from Chemical Engineering News, 12 June 1989. Copyright 1989 American Chemical Society)

Protease blocking substance used in HIV research

SmithKline Beckman claims to have developed compounds that slow the reproduction of the AIDS virus in laboratory tests. If the results can be independently replicated, drugs might be developed to prevent infected cells from releasing the virus and thus limit the spread of the disease. Human tests are at least a year away, however. The basis of SmithKline Beckman's strategy is the blocking of an enzyme called protease, which plays an important role in the reproduction of viruses. Researchers know of a number of substances that can inhibit the activity of the bare protease in a test tube, but have wondered if such compounds can enter the AIDS virus and the cells it infects. SmithKline Beckman researcher S. R. Petteway, Jr. says that while the compounds tested did not halt the production of new viruses, they did greatly reduce and eventually stop the production of certain proteins important to the structure and continuing reproductive capabilities of new viruses. The compounds used are peptides developed by SmithKline Beckman according to knowledge about protease and the compounds upon which it acts. (Extracted from Wall Street Journal, 7 June 1989)

Model developed to aid research

A cell culture model of neurological AIDS has been developed by researchers at Albert Einstein College of Medicine. The model might help scientists understand the neurological damage incurred by foetuses infected by their mothers. The foetal nervous tissue develops in culture much as it does in vivo, based on comparisons of the cell cultures with autopsies of foetuses aborted by HIV infected women. The study shows for the first time that HIV can directly infect and destroy brain cells. This has been inferred from observation of paediatric AIDS victims, who are almost always retarded. Facial features are also deformed in foetal AIDS victims, indicating a trauma to the tissues. (Extracted from Science News, 1 April 1989)

AIDS virus may lie hidden

HIV, the virus that causes AIDS, may go undetected for years in tests of an infected person's blood, according to a new study in California. The study of 133 homosexually active men, monitored since 1984, found several who did not make antibodies to the virus for up to 30 months after becoming infected. The standard tests for infection in people or in donated blood depend on the presence of antibodies to indicate that blood is infected.

David Imagawa at the medical school of the University of California at Los Angeles (UCLA) detected HIV in 31 of these men using very sensitive techniques for culturing the virus or for detecting tiny amount of its DNA. These tests are not commonly available outside research laboratories.

Four of these men seroconverted - began exhibiting antibodies to the virus - between 11 and 17 months after they had been infected. Blood taken from three of these men years previously showed that they had actually been infected between 23 and 35 months before seroconverting. The 27 other men with the virus did not develop antibodies by the end of the 30-month study period.

Imagawa's study, published in The New England Journal of Medicine, confirms several others that found "silent infection", says William Haseltine of the Dana-Farber Cancer Institute in Boston. A leading AIDS researcher, Haseltine writes in an accompanying editorial that the results "are both encouraging and disquieting".

"The good news is that the replication of HIV-I may be spontaneously suppressed ... in far more people than has been supposed." It is only when the virus starts replicating in a person's body that AIDS develops. Haseltine notes that drugs may be able to duplicate this suppression.

But the study "raises the sobering possibility that HIV-I infections may be transmitted by blood and organ donors who are silently infected", says Haseltine. Whether the virus can be transmitted sexually during this latency period remains an important but unanswered question. (Source: New Scientist, 10 June 1989)

Relative of HIV linked to thyroid disease

A virus related to HIV may be to blame for a disease of the thyroid gland. Researchers at the Middlesex Hospital and the Institute of Cancer Research in London, with colleagues in Barcelona, have detected DNA similar to that in HIV's genome in the thyroids of five patients with Graves' disease. They failed to find such DNA in cells derived from healthy thyroids.

Patients with Graves' disease produce antibodies to thyroid cells, causing the gland to release too much thyroid hormone. The thyroid also frequently enlarges, producing goitre, which may need surgery.

Franco Bottazzo and his colleagues from the Middlesex used probes derived from the genome of HIV to look for retroviral sequences in the DNA of cells cultured from thyroid tissue removed during such surgery. They performed similar studies on lymphocytes from the blood of the Graves' patients, on healthy thyroid tissue and on tissue removed from patients with cancer of the thyroid. They found

virus like DNA in the thyroid tissue of all five patients with Graves' disease. In three of the patients, the researchers also detected the sequences in some circulating lymphocytes. There was no sign of these segments of DNA in the other cells studied.

The scientists now plan to clone the DNA segment that the probe detected in order to determine its genetic sequence. This will allow them to confirm that it codes for viral genes. If the genes do prove to be viral in origin, they cannot have come from HIV because none of the patients was infected with HIV.

Graves' disease is an "autoimmune" disease, as are several other common diseases, including one type of diabetes, rheumatoid arthritis and multiple sclerosis. In such autoimmune diseases, the immune system behaves as it does when fighting a viral infection, but the attack is directed against the body's own cells.

This observation has led scientists to suggest that persistent viral infections are to blame for autoimmune diseases: some viruses can change molecules displayed on the cell's surface, so making it appear "foreign" to the immune system. Such changes also occur in some human autoimmune diseases, but no one has yet found evidence to link these changes to the presence of a virus.

Despite years of searching for suspects, researchers have failed to pin the blame for specific autoimmune diseases on particular viruses, leading some scientists to suggest that common viruses might trigger autoimmune attacks in a tiny proportion of the people they infect. No one has been able to explain why such a minute fraction of people should be affected, however, and Bottazzo believes it more likely that rare viruses that are difficult to isolate are responsible.

So far, researchers have isolated human retroviruses belonging to only two families: the human T-cell lymphotrophic viruses (HTLVs), which cause rare forms of leukaemia, and the human immuno-deficiency viruses. Recently, there have been reports that HTLV-1 is linked with multiple sclerosis. In animals, researchers have already linked viruses more closely with autoimmune disease.

The researchers found the virus like DNA in cells cultured from thyroid gland tissue, but this does not prove that the DNA was in the thyroid cells. In patients with Graves' disease, the thyroid gland is heavily infiltrated with lymphocytes that attack the thyroid cells. The DNA found in the thyroid tissue could easily have come from them. The fact that the probe also reacted with DNA from circulating lymphocytes in three of the patients with Graves' disease also lends weight to the argument that, as in the case of the HIVs and the HTLVs, this new retrovirus attacks lymphocytes. (Source: New Scientist, 27 May 1989)

## Research on bacterial genes

### TNT eating bacteria could clean hazardous explosives storage sites

The Los Alamos National Laboratory has found that bacteria that eat nitroglycerin and TNT could aid in cleaning up hazardous explosives storage sites. Micro organisms found near munitions plants have become tolerant to soil and water contaminated with explosives. The bacteria can live off usually toxic ingredients. Placing the bacteria on nitroglycerin that has seeped into soil could decompose the material into a safe residue in six months. (Extracted from Machine Design, 11 May 1989)

### Firefly factor makes colourful genetic marker

Bacteria such as Escherichia coli will glow in the dark if supplied with the gene for the firefly enzyme luciferase and a supply of its natural substrate, luciferin. Now Keith Wood and his colleagues at the University of California in San Diego, who were first to isolate the firefly gene, have created four different clones of E. coli that glow with four different colours. The new clones could prove very useful for studying the way genes work within living cells.

All bioluminescent insects use the same system of luciferase acting on luciferin, but different species emit different colours. Luciferase isolated from a particular species always glows with that species' characteristic colour, regardless of the source of the luciferin, so the wavelength of the light is a property of the enzyme, not of the substrate. To examine this phenomenon in more detail, Wood and his colleagues turned to the click beetle, Pyrophorus plagiophthalamus.

Click beetles have two lights, one on the top of the head and one at the front end of the abdomen, and are unusual because different individuals emit a wide range of colours. The headlamp is greenish, but varies from green at 548 nanometres to yellow-green at 565 nm. The abdominal light has a longer wavelength, but again varies from beetle to beetle, with colours between green (547 nm) and orange (594 nm).

Wood collected the beetles in Jamaica and froze them in liquid nitrogen. Back in the laboratory, the team isolated messenger RNA from the abdominal light organ and prepared complementary DNA copies of the RNA. Putting the DNA into the bacteria and washing luciferin over the colonies revealed a total of 11 clones of E. coli that had taken up click beetle DNA and were making luciferase.

The clones emitted one of four wavelengths: green 546, yellow green 560, yellow 578, and orange 593 nm. (Beetles in the wild emit other wavelengths within this range, so it is possible that there are other luciferase genes besides these four.) Wood sequenced the luciferase genes from four different colours and discovered that they all code for an enzyme 543 amino acids long. The exact sequence, however, varied slightly, by between 1 and 5 per cent among the four different genes. So it does not take many changes to the amino acid sequence of the luciferase to make it glow with a different colour.

Forty eight per cent of luciferase from click beetles is the same as firefly luciferase, but the differences are spread throughout the whole enzyme. This suggests that there are no particular regions that have to be maintained to enable the enzymes to work.

Molecular biologists have already used the gene for firefly luciferase to tell them whether other genes in the cell are working. If they put the firefly gene downstream of another gene, any light emitted will reveal that the other gene is operational. With four different colours at their disposal, they will be able to follow up to four different genes in one cell. (Source: New Scientist, 24 June 1989)

Salmonella's defence lies in one gene

A single gene may enable some bacteria to elude their host's defences, and so cause disease. Salmonella typhimurium is a bacterium that can cause food poisoning in people and a disease in mice that resembles human typhoid. It escapes immediate attack because it is immune to macrophages, which normally engulf and kill micro organisms as they invade the host.

Patricia Fields and her colleagues at the Scripps Clinic and Research Foundation in La Jolla, California, isolated three mutant strains of S. typhimurium. These bacteria, unlike the normal strain, could not survive inside mouse macrophages. An extract from rabbit macrophages killed these mutant bacteria quickly. The most effective ingredients in the extract were several small proteins.

Fields and her colleagues mixed the mutant bacteria with NP-1, one of these proteins. This is a defensin, a small peptide found in macrophages, that can kill bacteria in the laboratory. NP-1 killed the mutant bacteria, whereas the normal strain survived. The mutants were sensitive specifically to defensins, but not to other compounds produced by macrophages.

The researchers analysed the DNA of the mutant strains and found that all of them had a mutation in a gene called phoP. This gene is involved in producing an enzyme, non-specific acid phosphatase. When the researchers added copies of normal phoP to the mutants, they behaved like normal S. typhimurium, causing disease.

Other mutant strains that lack the enzyme are not, however, killed by defensins. So sensitivity to defensins is probably not the result of a deficiency of that enzyme. The researchers believe that phoP regulates other genes, including the gene that directly produces phosphatase and genes that control bacterial virulence.

PhoP is the first gene that scientists have found in Salmonella organisms that controls resistance to the host's defences. The mutant strains may help scientists to work out how normally defensins work. (Source: New Scientist, 1 April 1989)

Ice nucleating substance from Erwinia

Pseudomonas may not be the only bacterial strain capable of producing ice nucleating substances. Researchers at Kansai University (Suita City), led by Satoshi Obata, have identified a substance probably a protein secreted by cultures of Erwinia that Obata isolated from strawberry plants. The substance forms spherical particles 25 nanometers in diameter and causes ice crystals to form at temperatures greater than 0°C. (Source: Bio/Technology, Vol. 7, April 1989)

Bacterium with a vaccine in its tail

Researchers in California have found a new way to package a foreign protein into the tail, or flagellum, of a bacterium, in order to produce a potential vaccine.

Proteins isolated from an organism that causes disease may trigger an immune response from the body without causing illness, but the response may be weak or absent. The immune system in mice responds readily to the flagellum of a bacterium, so by incorporating a foreign protein into the flagellum, researchers may ensure that the system "notices" the foreign substance and activates its defences.

Saiete Newton and others at Stanford University School of Medicine in California have inserted part of the gene that produces a toxin in cholera into the gene that produces flagellin, a protein in the tail of a strain of salmonella. They used the cholera gene as a model for testing the response of the immune system of mice to the resulting "chimeric", or altered, flagellum.

The results were encouraging. The bacteria carrying the altered gene had functional flagella and could move easily. More importantly, protein made from the cholera gene was incorporated into these flagella. The immune system of mice recognized the foreign protein, or antigen, in the flagella and the mice produced antibodies to the cholera toxin. (Source: New Scientist, 29 April 1989)

Active growth factor expressed in bacteria

Active platelet-derived growth factor (PDGF) type BB can now be expressed in Escherichia coli, according to J. Hoppe, H. A. Weich, and W. Eichner of the department of cytogenetics, GBF - Gesellschaft für Biotechnologische Forschung mbH, in Braunschweig, Federal Republic of Germany. PDGF is a serum mitogen (a mitosis inducer) that promotes cell proliferation. Biologically active PDGF is a dimer in which homologous A and B chains are linked together by disulfide bridges in AA, BB, or AB configurations. Researchers have been able to produce biologically active dimers in a eukaryotic expression system, but attempts to express PDGF in E. coli have not led to active products. The researchers report the expression of recombinant PDGF B chain in E. coli and its renaturation into active PDGF type BB. (Reprinted with permission from Chemical Engineering News, 17 April 1989. Copyright 1989 American Chemical Society)

Cellulose bacteria

Bacteria that "fix" nitrogen from the atmosphere into nitrogen compounds are essential for the fertility of the soil. In order to fix the nitrogen, these bacteria need readily available sources of energy. One potential source is cellulose. However, biologists were unsure until recently whether it provided food for bacteria that fix atmospheric nitrogen.

Recent research by S. Leschine and his colleagues at the University of Massachusetts at Amherst has shown that four strains of bacteria involved in the breakdown of cellulose can fix nitrogen from the atmosphere. The bacteria were all anaerobic; that is, they did not require oxygen to function.

The researchers isolated the four strains from forest soil and freshwater mud and grew the bacteria in two liquid media, both of which lacked nitrogen compounds but included cellulose. The team exposed one culture to gaseous nitrogen and the other to an inert gas, argon. They found that much of the cellulose in the liquid exposed to nitrogen had gone after a period between seven and 14 days. The amount of cellulose exposed to argon remained unchanged.

Leschine's group also showed that the bacteria contained nitrogenase, an enzyme involved in fixing atmospheric nitrogen.

Many soils are rich in cellulose but contain few nitrogen compounds - for example, peat soils or municipal or agricultural waste sites. The researchers point out that it would be possible to make such soils much more fertile by adding to them these bacteria. (Source: New Scientist, 10 June 1989)

**Research instrumentation**

Computerized scanner faster and cheaper

Biolog (Hayward, CA) claims its new computerized scanner can identify a nearly infinite number of micro-organisms at a faster and cheaper rate than traditional methods. Biolog's system uses a microplate of 96 miniature wells, each filled with dye, nutrients and a different chemical. When exposed to a microbe, the wells turn different shades of violet. The computer examines the colour pattern and identifies the microbe present. Traditional methods used by microbiologists involve feeding microbes specific nutrients and then measuring the meal's effect on the acid content of the microbes' respiration. Identification is based on knowledge about various bugs' feeding likes and dislikes. The drawback is that the process is time-consuming and tracks only microbes that feed on carbohydrates. (Extracted from Wall Street Journal, 5 June 1989)

Protein BioEngine

The BioEngine is the most powerful computer system available for analysing, modelling and designing biologically active molecules and polymers. Such manipulation provides insights into the molecular basis of biological systems and chemical processes. Driven by GLOBAL, a fifth generation programming language and expert system, the BioEngine facilitates an interactive learning process between scientist and machine. The software has been written for the Norsk Data 5000 series of super micro-computers.

The technology for the BioEngine has been developed over the last 20 years, principally by Dr. Barry Robson and others who now form the heart of Proteus Biotechnology Ltd. Established in 1987, Proteus Biotechnology drew on funding from Imseco Medical Services. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Microscope reveals molecules at work

Advances in microscopy mean that scientists can, for the first time, watch biological processes in action at the molecular level. Using a new type of instrument, the atomic force microscope, Paul Hansma and his colleagues at the University of California in Santa Barbara have taken images of the blood-clotting protein fibrin as it polymerises.

The microscope, known as the AFM, is one of the latest developments in scanning probe microscopes. It uses a splinter of diamond to "feel" the surface of a material. A system of delicately sensitive cantilevers transmit and measure optically the deflection produced when the needle bobs up and down as it traces the uneven surface of the molecules.

The AFM is ideal for imaging biological molecules, such as proteins because it can scan surfaces directly and can also scan in water, fibrin's natural habitat.

Hansma recorded what happened to a solution of fibrinogen on a mica surface when he added a few drops of the clotting enzyme thrombin. Thrombin converts fibrinogen into the monomer of fibrin by removing sets of peptides from its centre. The monomers immediately start to join up to form polymers.

Hansma believes that scanning probe microscopes such as the AFM will revolutionize the study of biological and chemical processes. One problem is that the protein moves about in water. Hansma hopes to improve the quality of the images by lowering the temperature enough to keep the protein still, or by imaging the protein in more viscous fluids. (Source: New Scientist, 29 April 1989)

Images of DNA produced during gel electrophoresis

A new electrophoretic effect and associated technique - pulsed oriented electrophoresis (POE) - have been introduced by two scientists at Carnegie Institution of Washington's department of embryology in Baltimore. At the same time, the two scientists - Carnegie staff associate David Schwartz and John Hopkins University graduate student Michael Koval - have produced what they say are the first clear images of individual, fluorescently stained molecules of DNA as they migrate during gel electrophoresis. Traditional gel electrophoresis - most successful with small DNA molecules - separates molecules of DNA according to their sizes by running them through a gel-like matrix using a steady electric field. An electrophoretic system developed several years ago by Schwartz and called pulsed field electrophoresis took advantage of the distortion of large molecules that interact more frequently with the matrix by continuously reorienting the direction of an applied electrical field, as determined by molecule length. It thus became possible to resolve even chromosome-sized DNA molecules. POE, developed recently by Schwartz, is similar to pulsed field electrophoresis but uses pulses that are much shorter in duration - three to five seconds rather than one to two minutes. POE allows the separation of still larger DNA molecules. (Abstracted with permission from Chemical Engineering News, 17 April 1989. Copyright 1989 American Chemical Society)

**General**

STM measures RNA, DNA periodicities

Scanning tunneling microscopy (STM) has been used to measure the helical periodicities and to observe the alternation of major and minor grooves in double-stranded RNA and DNA molecules. Images of DNA have been reported previously, for example by Gerhardt Binnig and Heinrich Röhrer, of IBM's research laboratory in Zurich, Switzerland. These researchers won the 1986 Nobel Prize in Physics for inventing the STM technique. However, these images were either obtained at low resolution or were so distorted that no reliable information could be obtained about helix dimensions. Now, Gil Lee and colleagues from the departments of chemical engineering and biochemistry of the University of Minnesota have shown that nucleic acid dimensions and structural features can be quantitatively assessed by STM. (Reprinted with permission from Chemical Engineering News, 1 May 1989. Copyright 1989 American Chemical Society)

Malaria immunity

Experimentally, the best immunity to malaria has been achieved when irradiated sporozoites are used in vaccines, but this does not mean that the immune response is directed against the sporozoite

stage of the parasite. The malaria parasite has a complex life cycle: sporozoites are introduced into mammalian hosts in the bite of a carrier mosquito; these soon enter and mature in the liver; subsequently merozoites are released and invade red blood cells. One target of the immune response appears to be the parasite laden liver cell of an infected individual; this cell has now been found to display parasite antigens on its surface. Hoffman et al. of the Naval Medical Research Institute, Bethesda, MD, report that there are many inflammatory infiltrates in the livers of immunized animals and that among the infiltrating cells are cytotoxic T cells; this type of cell can recognize parasite antigens on liver cells in a genetically restricted fashion, probably in conjunction with specific major histo compatibility complex molecules on the liver cell surface and can kill the infected liver cells in an in vitro assay. These results suggest that all of the pre red blood cell stages of the parasite, and not just the sporozoite, should be considered as candidate antigens for antimalaria vaccines. (Source: Science, Vol. 244, p. 1023, 2 June 1989)

Novabiochem brings out three endothelin peptides

Endothelin, the most potent known vasoconstrictor peptide was first reported last year by Yanagisawa et al. Since then there has been a large amount of interest in this area. The original paper described the common porcine human sequence and endothelin (rat) was described by the same group. However Yanagisawa has now reported that the human genome contains three endothelin genes. One, corresponding to the human porcine sequence has been named Endothelin 1. Another corresponding to the rat sequence has been named Endothelin 3 whilst a third, hitherto unknown, sequence has been named Endothelin 2. Novabiochem has produced all three endothelin peptides as well as sarafatoxin S6b, a similar snake venom toxin. (Source: Manufacturing Chemist, June 1989)

Mutant mice overthrow accepted theory of nerve regeneration

A mutant strain of mouse has unexpectedly challenged one of the main theories about how nerves regenerate after injury. Normally, the part of the nerve that becomes detached from the cell body degenerates after the injury. The nerve fibres that supply the muscles, unlike those in the brain and spinal cord, are then able to regenerate.

But in the mutant mice, the detached part of the nerve fails to degenerate. Despite this, injured nerves regenerate just as fast as those in normal mice. The discovery, by researchers at the University of Oxford, challenges the previously held theory that the destruction of the detached part of the nerve is an essential prerequisite for regeneration. According to this hypothesis, such destruction would offer the regenerating nerve a clear route to its destination.

Ruth Lunn, Hugh Perry, Michael Brown and their colleagues at the University of Oxford have been studying the influence of the degenerative processes on the subsequent regeneration of nerve fibre. They first established the mechanism by which the degeneration of both nerve fibre and myelin occurs.

They found that macrophages invade the damaged area from the blood. These break down and engulf the disconnected tissue. Yet in the brain and the spinal cord, where fibres do not regenerate, these cells fail to arrive in the damaged area. (This is probably because the exchange of material from the blood supply to the brain is more strictly controlled than elsewhere.) So it seemed that macrophages held the key to the recovery of nerve fibres in the peripheral nervous system, maybe by offering the fibres a clear route through the sheath of Schwann cells.

But remarkable serendipity enabled the team to show that this is not so. While they were comparing the rates of regeneration of nerve fibres in different strains of mice, Lunn, Perry and their colleagues discovered a mutant strain in which normal Wallerian degeneration did not occur. Macrophages did not invade, myelin and nerve fibres remained intact, and Schwann cells failed to multiply. Yet fibres in the sciatic nerve of the mutant mice regenerated just as fast as those of normal mice.

The result has come as something of a surprise, not least to those who obtained it. They are now embarking on studies to show what path the regenerating fibres take through the surviving nerve stump in the mutant mice. (Source: New Scientist, 13 May 1989)

Parasite offers clues to its line of attack on the immune system

Many parasites suppress the immune response of their host, and researchers have long believed that the protozoan parasite that causes Chagas' disease, or American trypanosomiasis, may be among them. Researchers in the US have shown exactly how the parasite undermines the immune defences. They found that it interferes with a natural chemical produced by cells of the immune system.

The protozoan parasite that causes Chagas' disease, Trypanosoma cruzi, is common in domesticated and some wild animals. Researchers at George Washington University in Washington DC, and at Michigan State University found that the focus of the parasite's attack is interleukin-2, which acts as a "red alert" signal between the white blood cells known as T cells.

T cells encountering a foreign invader produce interleukin 2 which then stimulates other T cells. T cells are a diverse group which play several vital roles in the immune system. Without them, the immune response is far less effective.

An intriguing feature of the new findings is that T. cruzi can interfere with the interleukin 2 messenger in more than one way. When the research team studied the effects of T. cruzi on mice, they found that the parasite suppressed production of interleukin 2. But in human T cells, T. cruzi also blocked production of the interleukin 2 receptor molecule.

This is a protein that sits on the surface of T cells and can bind to interleukin 2 in the surrounding body fluids. When it binds to interleukin 2, the receptor stimulates the cell. Without interleukin 2 receptors, the T cells are deaf to the message that interleukin 2 carries.

The researchers also discovered that T. cruzi produces a soluble mediator which can suppress the production of the receptor. A drug which blocked its effects might improve the prospects for the millions affected by Chagas' disease but, the researchers warn, T. cruzi probably attacks the immune system in other ways as well. (Source: New Scientist, 6 May 1989)

Heat-shock proteins help you to "stay in the kitchen"

One of the most fascinating aspects of a cell's response to a heat shock is the development of tolerance to normally lethal temperatures. For example, mammalian cells, which normally grow at 37°C, are rapidly killed at 45°C. If cells in culture are given a sublethal heat shock by exposure to 43°C for an hour followed by several hours' recovery at 37°C, the surviving cells are tolerant to a subsequent heat shock at 45°C. Many will survive even higher temperatures.

Many scientists disagree about which heat-shock proteins are responsible for the development of thermotolerance. Some think that the hsp 70 family is responsible, others prefer to point the finger at the smaller heat-shock proteins. There are also those who maintain that heat-shock proteins are not required at all. This conclusion arose from the frequent observation that inhibitors of protein synthesis of hsp 70, for example, do not appear to block the development of thermotolerance.

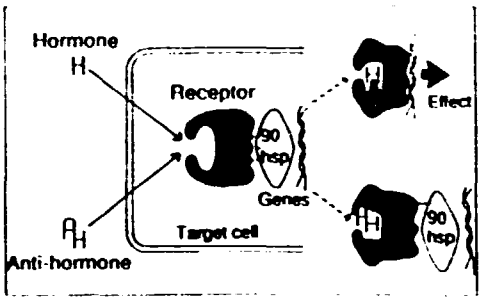
But thermotolerance merely provides an additional protection from damage by heat. The heat-shock proteins already present in the cells probably dictate the baseline level of resistance to heat. The additional resistance seen after the first heat treatment must be due to some additional factor synthesized by the cells. William Welch and Lee Mizzen at the Cold Spring Harbor Laboratory, in New York, have produced evidence that a potent inhibitor of protein synthesis, cycloheximide, does not block the development of thermotolerance, because it somehow stabilizes complexes called polysomes against heat-induced disaggregation. After a heat shock, these polysomes provide a head start in the resumption of normal protein synthesis.

There is also the possibility that thermotolerance results from a subtle redistribution of heat-shock proteins inside the cell (rather than their heat-induced expression per se). We already know that hsp 70 moves to the nucleus after a heat shock, but hsp 70 and 90 are also known to be associated with elements of the intracellular networks of filaments known as the cytoskeleton. These networks have important functions in almost all cellular events and, given the modulatory and protective roles postulated for heat-shock proteins, it is possible that the dynamic equilibrium of the cytoskeleton may be altered by heat shock. Its stabilization is an important (and until recently, overlooked) factor in the acquisition of thermotolerance. (Source: New Scientist, 1 April 1989)

HSP 90, the neglected heat-shock protein?

Most of the literature concerning the heat-shock response makes only a passing reference to hsp 90, ever though it is also inducible by heat. Most unstressed cells contain considerable levels of this protein, and hsp 90 from different species appears to be immunologically related. Its function is only now becoming clear.

Monoclonal antibodies, raised against intact steroid hormone receptors, cross-react with hsp 90. Experiments showed that hsp 90 binds away from the hormone-binding site itself. This inevitably led to the conclusion that hsp 90 masks the DNA binding site of the hormone receptors, until a hormone is positioned within the hormone binding site (see figure). When this happens, the hsp 90 is released, enabling the hormone receptor complex to bind to the DNA. In contrast, receptors exposed to anti-hormones cannot bind DNA and remain associated with hsp 90. So this heat-shock protein definitely does regulate the activity of steroid hormones.




Ill humour: heat-shock protein 90 regulates the action of steroid hormones by masking their normal DNA binding site.

Hsp 90 also turns up in the machinery of protein synthesis. It is a component in a complex set of factors involved in the control of protein synthesis. The precise function of hsp 90 in this system is still unclear, but it appears to modulate the phosphorylation, and therefore the activity, of the alpha sub-unit of "initiation factor 2" in animal and plant cells. Such modulatory activity is significant because the phosphorylation of proteins, or kinase activity as it is more commonly referred to, is of enormous importance in biochemical reactions. A cell's response to heat shock may thus be mediated by an hsp.

Finally, as with hsp 70, hsp 90 has an immunologically related counterpart, grp 94, located within the endoplasmic reticulum. Its role in this compartment is still highly speculative, but it is thought to be involved in the assembly of other proteins. (Source: New Scientist, 1 April 1989)

How diagnostic DNA meets its match

DNA probes set DNA to detect other DNA, and the basis of doing that is the hybridization reaction. The two strands of the DNA double helix are held together by weak interactions, called hydrogen bonds. If the two strands of the helix are separated, they will spontaneously come back together. DNA strands from different sources can form double helices in this way if the bases that make up the two strands fit into each other.

To form a stable helix, the four bases that are repeated in various patterns in the DNA must fit together correctly in space, so that the distance between the outer edges of the helix is constant and a regular helix can form. This means that, of the ten possible pairwise combinations of bases with each other, only A with T and G with C are allowed. So if one strand has the base sequence ACGTCCG, the other must have the sequence TGCAGGC opposite to it to form a double helix.

Whether a helix forms depends on what percentage of the bases are "matched" in this way - the smaller the fraction of matched bases, the less likely the helix is to be stable, because the fewer hydrogen bonds can be formed. Similarly, reducing the length of the single strands reduces the stability of the helix, as does altering a variety of reaction conditions - increasing the temperature,

for example, "melts" the helix apart. So a synthetic DNA can be tailored such that, under particular hybridization conditions, it will hybridize with only an exactly matched DNA, or with one that is within 5 per cent of exact matching, or within 10 per cent. Combined with knowledge of the likely variation in the DNA sequences of different genes in viruses and bacteria, this allows scientists to construct a probe that will hybridize to the DNA from a specific group of bacteria - one strain, one species, one genus - but not to any other. (Source: New Scientist, 6 May 1989)

Making a sandwich out of DNA

The technique known as sandwich hybridization uses two probes that hybridize to adjacent sections of the target DNA. One, the "capture probe", is fastened to a solid support so that when the target hybridizes to it, it is also linked to the support. The other, the "labelled probe", is linked to a reporter group - a fluorescent molecule, an enzyme or a radioactive atom. This probe will not hybridize to the capture probe, and so in the absence of target molecules the labelled probe will not stick to the support. If the target is present, however, it links capture probe and labelled probe, holding the labelled probe onto the solid support. If this support is a tube, washing the tube out will remove all the unbound labelled probe, so the amount left in it is a measure of the amount of target in the original sample. If the support consists of tiny plastic particles, microbeads, these can be separated by centrifugation or magnetic attraction, from the unbound labelled probe. The amount of the labelled probe remaining stuck to them tells how much target DNA there is. (Source: New Scientist, 6 May 1989)

Transition-metal complexes probe DNA conformation

A central challenge in molecular biology is to elucidate the mechanisms involved in site-specific recognition of deoxyribonucleic acid. Many proteins interact with DNA and, in one way or another, modulate its activity, a process that is responsible, ultimately, for regulating the structure and function of cells. Such DNA-binding proteins and enzymes locate and bind to a specific, small sequence of bases in the presence of an enormous number of other sequences, and then carry out a complex series of reactions at that site.

What are the principles that govern such site-specific recognition? The question is inherently a chemical question with important biological ramifications rather than a purely biological question, and chemists are using a variety of approaches to address it.

One such chemist is Jacqueline K. Barton, a chemistry professor at Columbia University in New York City. Barton has focused on the interactions of small transition-metal complexes with DNA as models for site specific reactions. To that end, Barton and Columbia co-workers have synthesized rigid, co ordinatively saturated metal complexes that recognize and bind to a variety of DNA sites based upon their shape.

The research suggests that DNA conformation is a critical element in biological site-specific interactions between proteins and DNA. A product of the work, which has been supported by the National Institutes of Health and the National Science Foundation, is a family of metal complexes that can be used to probe what Barton calls "the topology of DNA". Complexes have been developed that bind preferentially to several different DNA conformations such as Z-DNA, A-DNA, and cruciforms.

High-resolution crystal structures of oligonucleotides have revealed that three helical conformations of DNA exist: A, B, and Z forms. A-DNA and B-DNA are both right-handed helices, and Z-DNA is a left-handed helix. There are, in addition, a range of local variations within each helical family. And a number of unusual structures, which have not been characterized crystallographically, also appear to exist. One example of such a structure is the cruciform, a result of palindromic secuences of bases, which may extrude out from the normal duplex DNA.

A primary motivation of Barton's research is to determine whether this conformational heterogeneity has biological signficance. To determine where along the DNA strand a given molecule binds, Barton couples reactivity and site-specific binding, usually through a metal-mediated redox reaction that causes cleavage of the DNA strand at the bound site. (Abstracted with permission from Chemical Engineering News, 12 June 1989. Copyright 1989 American Chemical Society)

## D. APPLICATIONS

### Pharmaceutical and medical applications

Possible effective drug against HIV

GLQ223, derived from the root of a Chinese cucumber plant, appears to kill only those immune system cells harbouring the AIDS virus, according to Dr. M.S. McGrath of the University of California (San Francisco) and San Francisco General Hospital, and J.D. Lifson of Genelabs (Redwood City, CA). The extract of Trichosanthes kirilowii has been used in China since 300 AD to induce abortions, since the protein kills trophoblasts, cells of the placenta that resemble macrophages. It is the only drug tested against AIDS that destroys only AIDS-infected cells, ignoring other cells. It is also the only drug that deals directly with macrophage cells, which act as a reservoir for the virus in the body. AZT, the only drug now licensed for the treatment of AIDS, prevents AIDS from replicating in T-4 cells - immune system cells that are destroyed by the virus - but does not affect macrophages. GLQ223 also kills infected T cells.

The researchers did not disclose their findings for two years until they were ready to test it in people because they did not wish to raise false hopes in people with AIDS. McGrath discovered it about two and a half years ago when he was visited by Hin-Wing Yeung, a biologist from the Chinese Medicinal Materials Research Centre. McGrath and others warn that people with AIDS cannot obtain the highly purified drug by visiting China. Sandoz (Switzerland) helped finance the research and will have exclusive rights to market the product. (Extracted from New York Times, 18 April 1989)

Possible use of gene therapy

Research indicates gene therapy may be useful in preventing second heart attacks. The conclusion was made after two unique experiments on animals, one at the University of Michigan Medical Center (Ann Arbor, MI) and another at the Whitehead Institute and the New England Medical Center (Boston, MA). Gene modified cells in the University of Michigan experiment helped generate new smooth

linings in a pig's artery. In the Boston experiment, similar production of new blood vessel lining was done with dogs. (Extracted from Wall Street Journal, 27 June 1989)

Advances in vaccine development

Efforts to develop a genetically engineered vaccine to protect against strep throat and certain other bacterial diseases, such as gonorrhoea, have previously been hindered because of the many different forms the infection-producing protein can take. In strep throat, the M protein can categorize the streptococci into over 80 different "serotypes", each of which can produce an infection. Researchers at Rockefeller University (New York City) may have found a way around that problem. They recently identified portions of the M protein that are common to all the different serotypes that cause strep throat. That has allowed the researchers to design a vaccine in which this specific area on the protein is inserted into a carrier virus, making the vaccine capable of inducing immunity to different serotypes. Rockefeller University also says the findings represent the first vaccine against a bacterial disease that has been engineered using the vaccinia, or smallpox virus, as a vaccine transmitter. (Source: Chemical Week, 12 July 1989)

Malaria vaccine trials on people

A malaria vaccine developed by Ribi ImmunoChem Research (Hamilton, MT) and SmithKline & French Laboratories (Philadelphia) is beginning Phase I human trials under the sponsorship of the US Navy. The study, which will determine the safety of the malaria vaccine, will involve military volunteers and will last at least three months. (Source: Chemical Week, 12 July 1989)

Vaccines against food toxins

Oral vaccines might be able to protect people against carcinogens and toxins in food, according to D. Keren of the University of Michigan (Ann Arbor). The vaccine apparently stimulates the ileal immunoglobulin A to bind target substances, preventing their absorption by the intestine. Rabbit tests showed the technique works against absorption of the carcinogen 2-acetylaminofluorene. (Extracted from Medical World, 8 May 1989)

Possible AIDS vaccine developed

Researchers led by Dr. J. Salk have developed a vaccine that Salk claims may prevent persons infected with the AIDS virus from contracting the disease. Salk has been working for several years with researchers from the National Institutes of Health and the University of Southern California. He described his experimental vaccine at an international conference on AIDS held recently in Montreal, Canada.

While conventional vaccines are designed to prevent infection, the new Salk vaccine simply acts to keep an existing AIDS infection from progressing into full-blown AIDS. It consists of the AIDS virus stripped of its outer coating and killed by irradiation and chemicals. The vaccine also includes mineral oil to help trigger the body's immune system. Salk's experiments involved three chimpanzees, two of which were infected with the AIDS virus before being inoculated. All of the chimps were then given three doses of the vaccine over a period of several months. After this was done, the researchers waited 13-15 months and then injected more of the AIDS virus into each chimp. The two that had been vaccinated showed a strong immune response against the new infection, and eventually the virus disappeared from their systems. The researchers did admit that the virus could simply have been hiding in another part of the chimpanzees' bodies, but their tests did not detect it. The third chimpanzee that was not infected with AIDS before receiving the vaccine also showed a strong immune response after the second infection, but the virus remained. The quantity of virus present declined over a period of time, however. Salk has been testing his experimental vaccine in human volunteers since November 1987. No adverse reactions have been reported, but he adds that human subjects, unlike the chimps, still carried the virus after vaccination. (Extracted from New York Times, 9 June 1989)

Viral in AIDS vaccine trials

Viral Technologies is starting clinical trials of its AIDS vaccine at St. Stephen's Hospital, London. The vaccine, known as HGP 30, is different from other possible vaccines in that it is based on a copy of one of the internal proteins of the AIDS virus rather than an external envelope protein.

Laboratory studies using human cells have shown that HGP-30 stimulates the production of antibodies which stop the AIDS virus from replicating.

Viral Technologies, a joint venture between Cel-Sci and Alpha 1 Biomedicals, hopes that the same will be true in humans. HGP-30 also elicits a T-cell response from the immune system in vitro which scientists say is important in the development of a successful vaccine.

Other vaccines being tested in the US are based on an envelope protein which can change from one AIDS virus to another AIDS virus. Even if such a vaccine could protect against one virus there would be no guarantee that it could do so against others. (Source: European Chemical News, 15 May 1989)

Test detects cystic fibrosis in embryos

Molecular biologists in the Democratic Republic of Germany and the United Kingdom, working with embryologists and gynaecologists, have shown that it is feasible to diagnose cystic fibrosis in very young embryos. The tests can also diagnose Duchenne muscular dystrophy, another common genetic disorder in the West and, in theory, any disease caused by a single faulty gene. Soon, couples who know that they are at risk of having an affected child may be able to opt for such "pre-implantation diagnosis" after in vitro fertilization.

Charles Coutelle of the Central Institute of Molecular Biology in East Berlin, and Robert Williamson at St. Mary's Hospital in London, extracted DNA from a single human egg. They then used polymerase chain reaction to make many copies of the DNA sequence containing the cystic fibrosis gene. This gave them enough DNA to test for the disease in a matter of hours.

Their experiments provide a model for pre implantation diagnosis in embryos just a few days old and made up of between 4 and 16 cells. Their colleagues, Alan Handyside and Kate Hardy at the Royal Postgraduate Medical School in London, have already established that it is possible to

remove a single cell from such an embryo to extract DNA for testing, without damaging the rest of the embryo.

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Because the polymerase chain reaction quickly produces enough DNA for the tests, doctors could rapidly transfer into the mother's womb any embryo which was discovered to be free of the disease. This avoids having to freeze the embryo, and risk damaging it, while awaiting the results of the genetic test. (Source: New Scientist, 8 July 1989)

Hepatitis-C detection test

Chiron has developed a test to detect the hepatitis C virus, which is believed to cause most cases of non A, non-B hepatitis. The test could be used for blood screening and patient diagnosis. Chiron's test detected a virus in 17 of 24 cases of transfusion acquired non A, non B hepatitis and in 34 of 59 cases of unknown origin. The researchers say 78 per cent of chronic non-A, non-B cases in Japan and 84 per cent in Italy have tested positive for hepatitis C virus. Antibodies to the virus may take months to develop after infection. The new test kit uses viral protein to bind antibodies. A colour change reaction then makes the presence of the antibodies apparent. Many other cases of non-A, non-B may be caused by a mutant form of hepatitis B virus. (Extracted from Science News, 24 April 1989)

Test to diagnose type I diabetes

A genetic test to help identify children at risk of developing type I diabetes has been developed by M. Trucco of the University of Pittsburgh. The test is based on a flaw in a human leukocyte antigen protein among type 1 diabetics. Replacement of the usual aspartic acid in the protein renders it less capable of preventing immune cells from attacking pancreatic beta cells. People with the alteration are much more likely to develop type I diabetes than people without the alteration. The test, if accurate, could allow for early treatment of diabetes. Many children are not diagnosed until they suffer a diabetic coma. There is already an antibody test that can diagnose diabetes before symptoms appear, but only after beta cell destruction has begun. (Extracted from Science News, 10 June 1989)

Muscular dystrophy diagnostic available

Genica Pharmaceuticals (Worcester, MA) will introduce a test that can diagnose muscular dystrophy, an inherited muscle wasting disease that affects about 1 in 3,500 male babies. In 1986, researchers found the gene responsible for the disease. They also discovered that dystrophin, a protein made by the gene, reinforces muscle cell walls. Genica's test will detect levels of dystrophin in muscle cells, enabling doctors to diagnose the disease in newborns, before symptoms appear. With early detection and the ability to distinguish between different forms of the disease, the test may help prolong and improve the quality of life, a Genica spokeswoman claims. At Boston Children's Hospital, researchers are hoping to treat muscular dystrophy with injections of dystrophin. (Extracted from Wall Street Journal, 26 May 1989)

AIDS test marketed

Applied bioTechnology Inc. has introduced an AIDS research test to detect and analyse cells infected with human immunodeficiency virus (HIV-1). The test is the first non-radioactive test for AIDS that allows researchers to view and study HIV-infected cells.

Based on a technology known as in situ hybridization, the new test detects the genetic material in cells (nucleic acids) specific to the AIDS virus. The test format allows researchers to determine which and how many cells are infected, information not provided by either the antibody testing that currently dominates the AIDS diagnostic market or by newer antigen or nucleic acid tests. Unlike other nucleic acid tests for AIDS, the AbT test does not involve the use of radioactive probes.

AbT's test will be sold as a research kit primarily to institutions and pharmaceutical companies. It is the first product marketed by the company. In situ hybridization involves the detection and localization of specific nucleic acid sequences in cells. AbT's test employs nucleic acid probes comprised of all the HIV genes. When the probes are combined with cell samples, they bind to HIV RNA.

Infected cells which bind these probes are identified by colour, and the colour changes in these cells can be observed under an ordinary laboratory light microscope, providing a simple method of viral detection. AbT has enhanced the basic in situ technology to eliminate staining of non-infected cells. Because cells are fixed to slides and stained with a permanent colour, they can be preserved as a permanent record for future reference, whereas other HIV testing methods do not allow for such preservation.

The new test can detect as few as one infected cell in 100,000 and can be performed within 24 hours. It allows users to visually count infected versus non-infected cells and, unlike other HIV tests, it leaves cells intact, making it possible to identify the actual cells infected. (Source: Chemical Marketing Reporter, 3 July 1989)

Renin mapped

Canadian researchers have mapped the structure of renin, an enzyme that has an important role in the development of high blood pressure. A team from the University of Alberta, led by Dr. Michael James, found that the molecular structure of renin contains 340 different proteins.

In order to provide sufficient quantities of renin to enable the structure to be determined, scientists at California Biotechnology, Inc. and Dr. John Baxter of the University of San Francisco genetically engineered a synthetic version of renin.

Research is now continuing to find a substance that will inhibit renin and block its role in hypertension. (Source: Australian Journal of Biotechnology, Vol. 3, No. 2, April 1989)

Altered TPA may work better against clots

Protein engineers have altered the structure of a human enzyme - tissue plasminogen activator (TPA) - that is used to prevent heart attacks by dissolving arterial clots. The altered version of the enzyme is much less sensitive to its natural inhibitors, sparking hopes that it may turn out to be an improved anti-clotting agent.

Large doses of TPA are needed because its half-life in blood is so short. It not only is quickly cleared by the liver but also inactivated by various inhibitors, the most important of which is plasminogen activator inhibitor-1 (PAI-1). Because the drug does not persist, many patients develop new clots after treatment stops.

Seeking to overcome this deficiency, biochemistry professor Joseph F. Sambrook and four colleagues at the University of Texas Southwestern Medical Center in Dallas tinkered with TPA, removing a loop of seven amino acids. This structural change, they found, essentially prevents PAI-1 from inhibiting the enzyme. But the missing residues do not affect the enzyme's normal activity, which is to convert plasminogen, an inactive protein that circulates in the blood, into plasmin, a powerful enzyme that chews up blood clots.

It is still too early to tell whether the altered TPA will fulfil its potential as an improved anti-clotting agent. Sambrook's team is now conducting animal clearance tests to see if it works in animals as well as it does in the test tube.

Remarkably, the Texas researchers achieved their goal of blocking the TPA/PAI-1 interaction even though the three-dimensional structures of these proteins have never been deciphered. (Abstracted with permission from Chemical Engineering News, 3 July 1989. Copyright (1989) American Chemical Society)

Preventing the spread of tumours

The National Cancer Institute (NCI) has linked up with US Bioscience (Blue Bell, PA) to develop and commercialize a cancer treatment that could block the spread of tumours in the body. US Bioscience says this may be the first method to truly do so. Conventional treatments to check the spread of tumour cells include chemotherapy and hormonal therapy, but the firm says those techniques "do not always effectively discriminate between normal and cancer cells". Lance Liotta, NCI's chief of pathology and of the tumour metastases section, found that many cancer cells bind to a protein called laminin. Laminin lines cells in the body and provides a mooring for circulating tumour cells. If the binding sites are blocked with fragments of laminin, the circulating tumour cells cannot attach to the stationary laminin and grow. Another aspect of the treatment involves inhibition of the enzyme Type IV collagenase, which tumour cells secrete in order to invade healthy tissue. (Source: Chemical Week, 26 April 1989)

A skirmish in the virus war

Biotechnology firm Viagene (San Diego) says it is less than two years away from clinical trials of a genetically engineered treatment for viral diseases. The quest for treatments for viral diseases has been complicated in the past because of the inaccessibility of the virus's reproductive steps. Now Viagene is turning the virus's invasive nature against it. First the company alters a special carrier virus so it cannot replicate itself. Then Viagene inserts genetic material into the virus that instructs the target cell to take one of several disease-fighting actions. In the case of AIDS, for example, the cell will produce a surface antigen that helps the immune system detect and destroy AIDS-infected cells. The company has raised $8 million for further research, and expects to double that amount soon. (Source: Chemical Week, 5 July 1989)

Potential AIDS drug

A 20-year-old drug is gaining a new lease of life as a potential AIDS therapeutic. In vitro experiments indicate that the drug, known as GLQ223, blocks human immunodeficiency virus (HIV) production in T-lymphocyte cells and kills infected macrophage cells. Macrophage cells devour infectious agents and direct the attacks of the T-cells in the immune system.

Genelabs, a private biotechnology firm based in Redwood City, CA, received a US patent on GLQ223 early 1989. The firm submitted the drug to the Food and Drug Administration for the status of investigational new drug for treatment (IND). That title is given to promising drugs for lethal diseases and quickens their availability to the public.

GLQ223 is a highly purified compound - trichosanthin - derived from the root of a cucumber plant grown in China. There it is used to induce abortions and treat tumours of the reproductive system. But Genelabs found that it may also be effective against HIV infection, in a co-operative study with the Chinese University of Hong Kong and the University of California (San Francisco). Sandoz (Basel, Switzerland) will co-fund development and have exclusive world-wide marketing rights. (Source: Chemical Week, 26 April 1989)

Genetically altered cells tested in human patients

Genetically engineered cells have been injected into a cancer patient by researchers at the National Institutes of Health. White blood cells labelled with a marker gene from bacteria were injected into a patient with advanced melanoma. The tumour-infiltrating lymphocyte cells have shown promise in treating cancer, but are difficult to monitor inside the body. The bacterial gene responsible for resistance to the antibiotic neomycin was used as a marker gene, so that the distribution of the engineered cells in tissue could be monitored. The labelled lymphocytes will not provide any greater therapeutic benefits than unlabelled lymphocytes. The goal of the experiment is a greater understanding of the way that immunotherapy works, so that it can be improved. The study is an advance in the development of gene therapy for diseases such as cystic fibrosis, muscular dystrophy and sickle-cell anaemia. The study was delayed for almost one year pending receipt of Federal Drug Agency and National Institutes of Health approval. (Abstracted with permission from Chemical Engineering News, 29 May 1989. Copyright (1989) American Chemical Society)

Gene therapy breakthrough

Researchers at the Whitehead Institute of MIT have used gene therapy to take the first step towards correcting familial hypercholesterolemia - a life threatening human disease. Associate Professor Richard C. Mulligan and Dr. James Wilson inserted a foreign gene into a culture of liver cells from

rabbits and "cured" the defect. Although the work is preliminary, it could help pave the way for later application of gene therapy to many other diseases of the liver. (Source: Bio Technica Journal No. 2, 1989)

Drug developed to block cancer sites

A drug to block the site where metastatic cancer cells bind to basement membranes has been developed by researchers at the National Institute of Dental Research. The 5-amino acid compound attaches to laminin, a protein found in the smooth basement membranes that surround most organs. Mice injected with the new drug and 500,000 malignant melanoma cells developed only 10 per cent as many lung metastases as did untreated animals injected with the cancer cells.

The researchers have also developed lipoxygenase inhibitors that block the cascade of reactions needed by metastatic cells to allow them to cut through collagen found in the basement membranes. The inhibitors can help prevent the spread of ovarian carcinoma, which is highly metastatic. Each of the new drugs is non-toxic. with reversible effects. In some cases, the drugs might have to be used for life. In other cases, the drugs might be used only until surgery can eliminate a cancer. The drugs might also improve the effectiveness of traditional anti-cancer drugs, by preventing the metastatic cells from "hiding" in tissue. (Extracted from Science News, 15 April 1989)

New drug delivery systems

New drug delivery systems are being developed especially for proteins such as insulin, which are too large to pass through the skin, too digestible to be taken orally, or too unstable to be packaged in slow release mechanisms. Skin patches, nasal sprays, polymer microspheres, liposomes, etc., are being increasingly used to administer conventional drugs. At least 125 firms are attempting to develop delivery systems for recombinant proteins and peptides. TAP Pharmaceuticals has just introduced its Lupron leuprolide, a gonadotropin releasing hormone analogue, in a polymer based delivery system for the treatment of prostate cancer.

Eventually, physicians may be able to choose from a variety of delivery systems for every drug. Skin patches seem to be the most convenient form of drug delivery, offering pharmacologic benefits. But a molecular weight of 1,000 daltons is the upper limit for transdermal delivery. Insulin is 6,000 daltons, and most proteins are ten times larger than insulin. A low-level electrical current can increase the size of molecules that can be delivered transdermally. Ultrasound might also help transdermal administration. Transnasal delivery might take advantage of the relative ease with which compounds can traverse the mucous membranes of the nose. Transnasal insulin might be available by 1992. But dose control is difficult with transnasal administration. Eyedrops might also be a useful dosing route.

Polymers may offer a variety of administration routes. Polymers can be implanted, and can be made to dissolve gradually in the body. Implants might provide a constant dose level or a dose that responds to metabolic factors. Initial enthusiasm for liposomes and monoclonal antibodies as delivery agents has waned, although researchers are testing all the options. Liposomes might be useful for conventional drugs, but will be difficult to handle with protein and peptide drugs. And many of the protein drugs are tissue specific anyway, so they would benefit little from antibody linkages. (Extracted from Medical World, 10 April 1989)

Mab therapy in paediatric tumours

Some 30 per cent of paediatric tumours may respond to monoclonal antibody therapy, according to Nai-Kong Cheung of Memorial Sloan-Kettering Cancer Center. Antibodies against cell surface antigen GD2 have produced a 50 per cent tumour response rate in four of 13 neuroblastoma patients. The use of monoclonals in other cancers has not been very promising. The neuroblastoma cells may lack decay-accelerating factor that protects normal cells from complement-mediated death. Adding interleukin 2 might boost the response rate further. (Extracted from Medical World, 10 April 1989)

Antibody takes poison dart to lymph cancers

A new type of treatment for some cancers of the blood and the lymph glands may avoid most of the unpleasant side effects of many drugs used to combat cancer. Patients in Dallas and London are helping to test the therapy, which is a variant of the "magic bullet" approach of targeting drugs to attack cancerous cells and leave healthy cells intact.

The 20 patients all have cancer of the lymph glands of a type called B-cell lymphoma. The scientists who developed the therapy, from the Imperial Cancer Research Fund in London and the University of Texas in Dallas, say that equivalent therapies for T-cell lymphoma and Hodgkin's disease should soon be available for tests.

The treatment consists of an antibody linked to ricin. According to Philip Thorpe, who leads the team at the cancer charity, the poison is "outstandingly powerful". The antibody binds to the lymphoma cells, delivering a dose of ricin in the process. Because the armed antibody selects its targets, most of the patient's normal tissue is exposed to only low levels of the poison, and side effects should be fewer than with normal chemotherapy.

Thorpe and his colleagues wanted to direct the poison exclusively at tumour cells. They joined the A chain of ricin to an antibody molecule that recognizes only the body's B cells. The result, says Thorpe, is "almost perfect specificity for the cells we want to kill". Some healthy B cells at the same stage of development as the tumour do suffer, but the body regenerates these.

They are now testing the toxicity of the therapy in volunteers with B-cell lymphoma in whom conventional cell-killing drugs and radiation therapy have failed. Such failure occurs in more than half of patients with this cancer. There are about 1,500 new cases in Britain every year.

Peter Amlot, of the Royal Free Hospital in London, said the side effects produced by the therapy are nothing like those caused by cell-killing drugs, which include suppression of the immune system, anaemia, sickness and loss of hair. By contrast, the new treatment causes only a few temporary, minor side effects.

Thorpe and his colleagues say that they hope soon to have equivalent "poison loaded antibodies" to target malignant cells produced in the related diseases, T cell lymphoma and Hodgkin's disease. In the future, it might be possible to combine

conventional therapy with the antibody treatment to enhance the likelihood of cure. One characteristic of both Hodgkin's disease and both types of lymphoma is that the malignant cells they produce usually spread around the body in relatively small masses. An antibody linked to a poison can treat them effectively because it can penetrate the tumours more easily than it can large, solid tumours, such as those of the breast, lung and gut. Researchers need to develop ways of adapting their techniques to attack these solid tumours.

Such tumours have to induce blood vessels to grow in them. A tumour cannot grow more than 2 mm from the blood vessel - the distance that oxygen can diffuse - so tumours tend to grow cylindrically around blood vessels. Some researchers have calculated that each cell lining the tiny blood vessels in a tumour must supply 20,000 tumour cells with oxygen.

Francis Burrows, working with Philip Thorpe at the fund, is trying to find ways of killing specifically the cells lining the blood vessels inside the tumour. Burrows is growing these so-called endothelial cells in the laboratory and stimulating them with substances produced by tumours. He says that a variety of substances produced in the tumour, such as interferon, can alter the molecules on the surface of endothelial cells, and make them recognizable to a specific antibody. (Source: New Scientist, 1 April 1989)

Colgate to evaluate Synergen periodontal product

Synergen, Inc. has signed an agreement with the Colgate-Palmolive Company to evaluate Synergen's technology for the prevention of periodontal disease. Synergen's proprietary "adheson" technology is being developed to target therapeutic products to oral surfaces.

Gingivitis and periodontal disease are severe and extremely common inflammatory conditions. With the aid of grants from the National Institute for Dental Research, Synergen's scientists are using recombinant DNA technology to create novel compounds believed capable of eliminating or preventing the formation of dental plaque. If successful, these compounds could be incorporated into toothpaste or other over-the-counter products.

The agreement calls for Colgate to test model compounds developed by Synergen in Colgate's pre-clinical models of periodontal disease and to share the results with Synergen. For its work and for agreeing to provide additional financial support for Synergen's research, Colgate will have the option to negotiate a licence for Synergen's technology and to support further development work by Synergen. (Source: Company News Release, 10 May 1989)

Arthritis licence deal

British Bio-technology has signed a licensing agreement with SmithKline Beckman Corp. for the development and commercialization of collagenase inhibitors as new treatments for arthritis.

The human enzyme collagenase is a protein responsible for bone and cartilage destruction in the joints of sufferers of rheumatoid and osteo arthritis.

The two companies have been engaged in collaborative arthritis research since 1987. British Bio said the new agreement covers the development and marketing of potential anti-arthritis drugs arising from this joint research.

In this latest collaboration, British Bio's chemistry department has been designing and building specific collagenase inhibitors with the properties of these potential new drug molecules being tested at SmithKline's pharmaceutical laboratories in Upper Merion, Pennsylvania, USA. (Source: Manufacturing Chemist, April 1989)

New test can predict rejection of transplanted organs

Doctors can now predict which patients who have had organ transplants are about to reject the r grafts. The new-found ability to monitor the immunological response to a transplant also makes i easier to judge a patient's need for the immunosuppressive drug cyclosporin.

The discovery, by researchers at the Newcastle-upon-Tyne Transplant Centre in the north of England, makes it possible to measure the rejection process scientifically. Patients frequently succumb to rejection before they or their doctors notice that anything is amiss. As a result, researchers have tried to relate the behaviour of elements of the immune system to episodes of rejection.

Initial studies looked at levels of the protein called interleukin-2, which plays a role in the process of recognizing foreign tissue and rejecting it. This molecule, secreted by T-helper lymphocytes, activates another type of lymphocyte, the cytotoxic T cell. These cells assist in rejecting the graft, a process known as cell-mediated graft rejection.

Levels of interleukin 2 have not, however, proved a reliable indicator. John Forsyth at Newcastle decided to extend the research to the receptors for interleukin 2 which develop on the T lymphocytes when levels of interleukin 2 rise. The lymphocytes then shed, or secrete, these receptors.

Tests to measure the interleukin-2 receptors can give very accurate results, as Forsyth and his colleagues reported at a recent meeting of the British Transplant Society. The researchers recently tested 23 patients who had had a transplant, taking 300 samples over 14 days. In all but two cases, a high or rising level of interleukin-2 receptors indicated that rejection had begun or was about to begin.

The two "false positives" arose because one patient had an infection not associated with the graft, and another had active autoimmune disease which caused the same effect as rejection of the transplant. The researchers now intend to carry out double-blind trials to test the ability of the method to predict rejection.

Confirmation of the link between interleukin 2 receptors and rejection has a double benefit: it will also make it possible for doctors to tailor the doses of immuno-suppressive drugs they prescribe to a patient's needs. (Source: New Scientist, 3 June 1989)

Toxic sensor

Research at the University of Texas at San Antonio (UTSA) may benefit groups as diverse as soldiers, people living near chemical plants and Alzheimer's victims. Drs. Matthew Wayner and

James Chambers are developing a biosensor - an enzyme which acts as an instrument that would detect toxic substances in the air or even in the body. In this way, poisons that might be accidentally inhaled or swallowed could be detected and avoided. The biosensor would also alert us to viruses in the body even before symptoms appear. The researchers have received encouragement from the army, which is interested in how a biosensor could warn soldiers of the existence of toxins from chemical weapons. Much of the research also involves the early detection of Alzheimer's. Wayner and Chambers are working with enzymes from animal brains, monitoring reactions from poisonous substances.

For more information contact Ellen Sterner or David Bernert, Dublin-McCarter & Associates. (Source: BioBytes, May 1989)

Synergen initiates wound healing clinical trials

Synergan, Inc. has announced it will begin human clinical trials to evaluate its basic fibroblast growth factor (bFGF) for the treatment of topical ulcers. Synergen believes its clinical trials will be the first to investigate the role of bFGF in wound healing.

Phase I testing will focus on the safety profile of the compound. Phase II testing, scheduled to begin later this year, will examine the efficacy of Synergen's product for selected indications, including decubitus ulcers (pressure sores) and venous stasis and diabetic leg ulcers. These wounds, which afflict many elderly and bed-ridden patients, are difficult to heal with current therapies.

The human protein was first purified by Synergen's scientists working in collaboration with an academic group. It is currently produced using recombinant DNA techniques in Synergen's pilot plant. Over the past several years, Synergen has established production methodology meeting current FDA standards, developed uniquely stable formulations, and completed toxicological and animal efficacy testing of this compound.

bFGF is an extremely potent stimulator of angiogenesis, the formation of new blood vessels. It belongs to the general class of cellular growth factors and induces the proliferation of several cell types necessary for tissue repair including both fibroblasts and vascular endothelial cells. The human protein is also being evaluated as a potential treatment for cardiovascular conditions and, because it promotes the survival of neurons within the central nervous system, for the treatment of neurodegenerative disorders. (Source: Company News Release, 10 May 1989)

Synergin and Hoffmann La Roche announce anti inflammatory drug research and development collaboration

Synergen, Inc. of Boulder, CO, Hoffmann-La Roche Inc. of Nutley, NJ, and F. Hoffmann-La Roche & Co. Ltd. of Basle, Switzerland, have announced the signing of agreements to jointly develop IL 1i, interleukin 1 inhibitor, a newly discovered human protein. IL 1i, believed to be a central regulatory element controlling the inflammatory response, will be investigated as a potential treatment for several diseases, including rheumatoid arthritis.

An important part of the collaboration involves the use of structural and biological information about IL 1i to identify and design orally active analogues of the protein. Roche researchers are actively pursuing these second-generation products, which have potential for the treatment not only of arthritis, but of many other inflammatory diseases. It is too early, however, to predict their safety and efficacy profiles. Synergen's royalty interest will extend to products emerging from this programme. (Source: Company News Release, 22 May 1989)

**Livestock applications**

Tapeworm succumbs to engineered vaccine

Researchers in New Zealand and Australia say they have developed the world's first effective vaccine against a parasitic disease using techniques of genetic engineering. Most vaccines protect against viral or bacterial infections.

Developing a vaccine against a parasite - in this case a larval tapeworm - is difficult, because they are multicellular organisms which elicit a complex immune response in the host. In addition, parasites have evolved clever means of avoiding the host's immune responses by changing the structure of their antigens.

The new vaccine has been developed against the tapeworm parasite, Taenia ovis, which causes a disease in sheep commonly known as sheep measles. The name is derived from the spotty cysts that form in the sheep's muscles: the cysts resemble measles in appearance.

In trials in New Zealand, the new vaccine has proved to be effective against the disease 95 per cent of the time. Sheep develop the disease when it is passed on to them from dogs. The sheep ingest parasitic eggs of the tapeworm from dog faeces. The sheep industry in Australia and New Zealand is expected to save millions of dollars as a result of the vaccine - meat from sheep which are infected with the disease cannot be exported. Instead, the meat is destroyed.

A company called Coopers Animal Health is developing the vaccine for commercial use and expects the vaccine to be ready within two years.

Researchers from Coopers, the University of Melbourne, and New Zealand's Ministry of Agriculture and Fisheries have discussed the possibility of developing vaccines against other tapeworms that cause disease in both animals and humans. The diseases include beef measles, pork measles, and hydatids. These diseases are more wide-spread than sheep measles, and the development of vaccines for them will have applications all over the world.

The hydatid parasite, an adult tapeworm in dogs, can cause large cysts in the human brain, lungs, kidney and liver. It is prevalent in China. Sheep act as the intermediate host. People can also be infected by the pork measles parasite, Taenia solium. The parasite is common in Indonesia and Mexico, where it can cause a disease called neurocysticercosis. Humans carry the tapeworm Taenia saginata, which can cause beef measles in cattle. The parasite, passed on when cattle are exposed to human faeces, is a problem for the beef industry in South America and Africa and has been found in animal feed in the US. Researchers expect the work with sheep measles to help them to develop vaccines for these other diseases, because they believe that the immune response is similar. (Source: New Scientist, 15 July 1989)

Recombinant poultry vaccine

Applied Biotechnology (Cambridge, MA) and Genex (Gaithersburg, MD) have announced joint development agreements with the Hoechst Group to complete work Genex has begun on developing a recombinant vaccine against coccidiosis in chickens. Coccidiosis is a parasitic disease causing world wide losses estimated at nearly $1.8 billion. Hoechst will work with Genex to identify the antigens that protect chickens against this disease, and Applied Biotechnology will work with Genex to express these antigens in a novel viral vector system, with funding from Hoechst. (Source: Chemical Week, 3 May 1989)

Embrex and University of Arkansas on poultry virus neutralizing factors

Embrex, the US poultry biotechnology company, is to collaborate with the University of Arkansas on the development of substances which will protect poultry from viral infections. One such substance, known as a viral-neutralizing factor (VNF), was recently discovered by researchers at the University of Arkansas. Studies have shown that chickens given the VNF at the time they are exposed to infectious bursal disease virus or infectious bronchitis virus are protected from infection. The VNF has also been found to be active against certain bacteria and fungi. (Source: Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

Vaccine field test

Researchers at the Wistar Institute (Philadelphia, PA) are feeling frustrated in their efforts to field test a genetically engineered rabies vaccine. Officials at the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) concluded that the vaccine field test "would not have a significant impact on the quality of the human environment". Nevertheless, their approval was being held up by state officials in South Carolina, who have raised objections to the plans. Moreover, although the situation is slightly different at an additional proposed test site in Virginia because it is privately owned, neither state officials nor the owners had approved the test by early May.

The Wistar plans call for testing the engineered vaccine in wild animals occupying several small islands off the coasts of South Carolina and Virginia. Time was working against the Wistar scientists because they had to begin setting out vaccinia treated, orally administered anti-rabies baits sometime in May to allow completion of the protocol before water fowl hunting season begins in the fall.

The genetically engineered vaccinia virus carries no risk of causing rabies. APHIS officials point out that they have carefully analysed the protocol Wistar submitted and found it satisfactory. The experimental vaccine developed by Hilary Koprowski, Charles Ruprecht, and their colleagues at Wistar contains a copy of the gene for the surface glycoprotein of the rabies virus. Because that gene is inserted into the thymidine kinase gene of vaccinia, the virus is further attenuated. In laboratory tests, exposure of many animal species to this version of the antigenic glycoprotein confers resistance to rabies. Moreover, it "is non pathogenic, safe and efficacious in ... mice, hamsters, rats, and a number of target and non target species, including the major terrestrial wild life reservoirs of rabies", according to the APHIS assessment.

The field tests call for vaccinating wild animals with a preparation that is orally administered in specially treated baits. Officials in South Carolina said that the engineered vaccine, known as the Copenhagen strain, might pose a threat to public health. The health officials cite the strain's tendency to cause side effects, including encephalitis, when used to vaccinate humans. Such use in Europe was discontinued several decades ago.

Many experts familiar with current research on vaccinia viruses and the historical use of the virus in preventing smallpox deem such a threat unlikely in the context of the proposed field trial.

Moreover, in the planned field trial, it is unlikely that humans will be exposed to the vaccine at all, let alone by inoculation. The island test sites were chosen in part to minimize chances of human contact with the engineered vaccine. The vaccine is to be administered to animals orally when they consume a specially designed fish meal containing bait. (Extracted from Bio/Technology, Vol. 7, June 1989)

## Agricultural applications

Molecular farming route to peptides

Belgian plant biotechnology company, Plant Genetic Systems, in collaboration with the University of Ghent, has genetically modified plants so they can produce pharmaceutically important peptides. Dubbing the technique "plant molecular farming", PGS managing director, Walter De Logi, believes the economic potential for drug companies is significant.

With the technique, economically important peptides can be produced and stored in a stable form in specific plant organs, such as seeds. Products are produced at high levels and can be extracted by a simple process. Those so far produced include pharmaceuticals such as blood factors and growth hormones.

De Logi explained that the company plans to genetically engineer plants, grow the crop, extract the product, complete the first stage purification and then sell it to pharmaceutical companies. (Source: Manufacturing Chemist, April 1989)

Biotechnology tests pursued

Monsanto Company has begun new research field trials of tomato plants genetically engineered to resist certain insects, as well as tests of canola oil seed genetically engineered to tolerate "Roundup" herbicide.

The trait for "Roundup" herbicide tolerance has the potential to significantly reduce the cost of growing canola by allowing weeds such as wild mustard and stinkweed to be more efficiently controlled, Monsanto says.

Similar research field trials with canola were successfully conducted last year in both Saskatchewan and Alberta.

The field trial is being conducted at Scott Experimental Farm in west central Saskatchewan. Engineered and control canola seeds were planted and the crops will be studied for comparison and for weed control.

Canola, a type of spring oil-seed rape developed by Canadian researchers, is used primarily for cooking and salad oil, and in margarines and shortenings. Nearly 7 million acres of canola are planted annually in Canada, with the value of the crop estimated at $1.25 billion, according to the Canola Council of Canada.

Monsanto researchers placed a gene from another plant species into canola, instructing it to make extra quantities of an essential enzyme it already produces - EPSP synthase. In non-engineered plants, this essential enzyme is inactivated by "Roundup" herbicide, which causes the plants to die.

In the genetically engineered canola, the extra quantities of the enzyme and its decreased sensitivity allow the plants to grow normally despite the presence of "Roundup".

Monsanto planted insect-resistant tomato plants earlier this year in Mexico and Florida. (Source: Chemical Marketing Reporter, 29 May 1989)

Biocontrol agent is patented

Bio-Technology General (New York City) has been issued a US patent for its method of growing certain strains of trichoderma, which the company says could become a novel, non-chemical fungicide. The species, the company says, represents biological control agents capable of protecting commercially vital plants, roots and plant seeds from certain fungal diseases. The company notes that results from field trials have indicated that these species are ecologically safer than chemical fungicides. Bio-Technology General is currently looking for corporate partners to commercialize trichoderma. (Source: Chemical Week, 12 July 1989)

Early detection for crop diseases

Agri-Diagnostics Associates (Cinnaminson, NJ), a joint venture between DNA Plant Technology (Cinnaminson) and Beazer Materials and Services (Pittsburgh), has come out with a test kit for on-site detection of fungal disease in soybean and other crops. The tests detect disease pathogens including Phytophthora, Rhizoctonia, Pythium and Sclerotinia in crop plants. Also, a new 10-minute test kit to detect fungal disease in turfgrass is now available, replacing the previous kit that took three hours to give results. Steve Banegas, general manager of Agri-Diagnostics, says the firm is working with Ciba-Geigy (Ardsley, NY) on a soil assay for Phytophthora, to determine its presence in a field before soybean crops are planted. (Source: Chemical Week, 28 June 1989)

Potatoes produce soy protein

Kirin Brewery has developed a potato plant that can produce a soy protein in its leaves and tubers. The potato plant was modified to make glycinin with the aid of the National Research Institute of the Ministry of Agriculture, Forestry and Fisheries. The gene was introduced into the potato via an agrobacterium. Glycinin is rich in the essential amino acid lysine. Kirin had previously succeeded in engineering potatoes with antibiotic tolerance. (Extracted from Japan Chemicals, 11 May 1989)

Field trials of genetically modified potatoes

Shell Research Ltd. is carrying out small scale field trials this year on genetically modified potatoes, as part of a programme designed to develop crops with enhanced resistance to pests and diseases. Potatoes of the variety Desiree have been modified by the transfer of a gene from another edible crop plant. The gene is expected to confer an increased resistance to insect pests. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Tobacco plants produce desired proteins

Tobacco plants can be made to produce desired proteins by spraying them with an RNA-carrying vector, according to Biosource Genetics (Vacaville, CA). The RNA molecules are packed in a protein coat, much like a virus. The particles enter plant cells through a cut in the leaves, and the RNA starts to direct protein assembly in the cytoplasm, without entering the nucleus. The technique could be used to produce interleukin-2, interferon, serum albumin, etc. The technique might be field-tested in about a year, but US Environmental Protection Agency and US Department of Agriculture approval will be needed before the technology can be commercialized. (Extracted from Science News, 15 April 1989)

Early flowering tobacco

Researchers in Hirobumi Uchimiya's laboratory at Tsukuba University (Ibaraki) have constructed a strain of tobacco that flowers up to one month earlier than usual. Uchimiya had previously identified the gene that induces early maturation - the first example of its kind - as responsible for causing the plants to produce shorter stems.

The scientists isolated the early-maturation gene from an Ri plasmid harboured by a strain of soil bacteria that infects tobacco plants. They introduced the gene into tobacco plant protoplasts and measured the maturation rate of tobacco plants regenerated from the protoplasts. Of the 35 regenerated plants, most flowered three weeks - and some even a full month - sooner than the controls.

The development of rapidly maturing, sturdy strains would improve yields for many commercially important plants. To this end, Uchimiya and his colleagues plan to insert this gene into other plant species. (Source: Bio/Technology, Vol. 7, June 1989)

Agracetus begins field test of insect-resistant cotton

The world's first field test of genetically engineered cotton plants began on 27 April. Agracetus, a joint venture of Cetus Corp. and W.R. Grace & Co., received permission from the Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture to test the cotton, which Agracetus scientists had genetically modified to resist attack by damaging caterpillars such as the tobacco budworm and cotton bollworm.

The new cotton contains a gene from Bacillus thuringiensis, a common soil bacterium. The bacterium is harmless to other insects, man and animals, and has been widely used in biological pest control since the early 1960s. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

Allelix achieves first corn plants from pollen cells

Scientists at Allelix Crop Technologies of Ontario, Canada, have grown breeding (homozygous) plants from immature male corn cells. The

microspore-derived cell lines can be used to produce genetically engineered plants and improved hybrids. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

"Insect Exocet" could replace chemical pesticides

The UK's first bio-control product for black vine weevil has been launched by Agricultural Genetics Company (AGC). The weevil's larvae cause damage to glasshouse crops such as cyclamen by attacking the plant's main feeding roots. Because this attack happens below soil level, infestation may pass unnoticed until growth slows and leaf yellowing and wilting become apparent, by which time it is too late to save the plant.

In the past, control has been possible only by using the chemical aldrin, but the UK Government's recent legislation banning sales of this chemical from 1990, and its use from 1992, has heightened the need for an environmentally safe alternative. Nemasys, which will be marketed by AGC's Microbio Division, employs a nematode whose potential was first identified at the AFRC Institute of Horticultural Research by a group led by Paul Richardson.

The species carries a type of bacteria that is deadly to many insects and their larvae. The nematode has been nicknamed a "biological Exocet", because of its ability to seek and destroy insect larvae. It enters their bodies and releases its fatal load of bacteria. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

Monsanto developing plant vaccine

The inoculation of plants with a coat protein can confer virus resistance, according to Dr. Keith O'Connell of Monsanto Co. As a new approach for virus resistance, Monsanto researchers inserted into plants the gene from a virus which directs the production of the virus "coat" protein. The presence of the coat protein in the plant cells prevents the virus from infecting the plant.

To date, resistance to tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), alfalfa mosaic virus (AlMV), cucumber mosaic virus (CMV), tobacco streak virus (TSV), potato virus (PVX) and potato virus Y (PVY) has been introduced into corresponding crop plants.

Coat protein levels detected in the plants ranged from 0.002 to 0.1 per cent of total leaf protein. The average levels of virus detected in the plants with the added gene were 9 36 per cent of levels found in the control plants without the gene. Virus-resistant crops are not expected to reach the market until the mid 1990s. (Source: Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

## Food production and processing

Biotech at home

Using the same immunoassay technology now found in home pregnancy test kits, biotechnology companies will soon be marketing kits allowing consumers to check for contaminants in fruits, vegetables and drinking water, according to Consulting Resources Corporation. The consulting firm estimates that the market for such monitoring units could exceed $100 million within the next ten years. (Source: Chemical Marketing Reporter, 3 April 1989)

A cultural revolution for cheese

Biotechnologists are developing new techniques to mass produce cheese. In the US and European Community, some 50 billion litres per year of milk are now transformed into cheese. New techniques include genetic probes to detect and identify bacteria that can spoil foods or cause disease. Antibodies could also be used to detect contaminants. Researchers are also developing altered enzymes for cheesemaking. In addition to studying ways to control the curdling of milk, researchers are attempting to speed the maturation of cheeses, which is a complex biological process in which proteins and fats in the milk are converted to flavour compounds. Even the relatively simple process of splitting fats into fatty acids is difficult to speed up. Adding enzymes at the wrong time can spoil cheese. Encapsulation of the enzymes may provide some help. (Extracted from New Scientist, 27 May 1989)

Milk coating could push frozen foods into the cold

The dull fare of frozen pizza, chips and other convenience foods may soon become obsolete, following the development of a new technique to keep sliced fruit and vegetables fresh and tasty for several days. Instead of filling up shopping trolleys with the usual frozen assortment, people may soon be able to buy fresh food that has already been washed, sliced and pitted.

Attila Pavlath, from the US Department of Agriculture Research Service in Albany, California, has found a way to keep sliced fruit and vegetables fresh for up to three days by covering them with an edible coating based on milk.

Although farmers use coatings to increase the lifetime of their produce, until now no one has found a way of preserving fruit and vegetables that have been cut or peeled. The problem researchers face is to develop a coating that will stick onto a moist surface and prevent oxygen passing in and water passing out.

Most fruit and vegetables give off carbon dioxide after they have been picked. Unless the coating enables the carbon dioxide to pass through it, the taste of the food will change. The coating also has to be edible and perhaps the most difficult condition to meet - acceptable to the US Food and Drug Administration (FDA).

Pavlath took milk as a starting point because the proteins in it make a very good film. Protein films form an inadequate barrier against water and oxygen, so Pavlath had to modify them by adding enzymes to the milk.

These enzymes "tie" the proteins together. Pavlath used the technique to weave proteins together, so forming a mesh fine enough to reduce the amount of oxygen and water molecules crossing the barrier.

The films could also be used to modify produce and, according to Pavlath, add "exciting new flavours or colourings to standard fare".

One possibility, he suggests, is to "jazz up" sliced pears, by protecting them with a protein coating flavoured with natural red cherries. The coatings could also be used to keep fillings in pies and pizzas from soaking the crust, says Pavlath.

Although the barrier keeps cut fruit and vegetables fresh for several days, food companies are unlikely to use Pavlath's coating unless it can preserve food for several weeks, because of the amount of time it takes to distribute food to supermarkets.

Pavlath believes that he will be able to make his coating more effective by making the molecular mesh even finer. The problem, however, is not trivial, because if the mesh is too fine, then the film will become rigid and prone to crack, allowing the food to decay. (Source: New Scientist, 17 June 1989)

Bacteria identification

Biolog (Hayward, CA) have developed a process that identifies bacteria from the chemicals they consume. The company calls the technology "breathprints", and believes it may assist in detecting disease or contaminants in cheese or winemaking. Biolog has created a culture plate with 96 wells, one each for the 96 different chemicals consumed by so-called gram negative bacteria, which cause such diseases as pneumonia, gonorrhoea and influenza. Samples of unidentified bacterium are placed in each of the wells. If the bug metabolizes the chemical, a dye in the chemical turns purple. By examining the pattern of wells that have turned colour and comparing it to patterns produced by known bacteria, a computerized instrument can determine which specific bug is present. (Extracted from Business Week, 29 May 1989)

## Energy and environmental applications

Early warnings of stress

All kinds of cells and organisms, from bacteria to people, respond to harmful environmental conditions by synthesizing stress proteins, also known as heat shock proteins. As the severity of the stress increases, so does the production of the proteins. They appear in response to a host of events such as high temperature, lack of oxygen, and exposure to heavy metals, ethanol and, in mammals at least, to physical trauma.

Researchers know of at least 30 stress proteins, identified from the mass of cellular proteins separated by gel electrophoresis. Cells make them in varying amounts, depending on the type and severity of stress. The functions of the proteins are still unclear. Preliminary evidence suggests, however, that those made by an organism under stress protect it from damage that further exposure would cause.

Whatever their function after stress, the appearance of extra heat-shock proteins is a sure sign that the organism has been stressed. Biologists might be able to use this knowledge in at least three ways: to measure stress or pollution in the environment; to improve medical diagnosis and therapy; and in biotechnology. In each case, there is a commercial application that is about to reach the market place.

Current methods of measuring pollution in the environment have limitations. It is not easy to assess the severity of contamination by sampling and testing for various chemicals. In water, for example, the heavy metal cadmium may be chelated in a form that renders it harmless; measuring cadmium levels alone cannot distinguish between safe and toxic forms of the metal. Alternatively, measurements may indicate that the level of a heavy metal in water is low, but it may accumulate to toxic levels in the tissues of organisms, such as the livers of fish.

Williams Welch, a cell biologist at the University of California at San Francisco, thinks that stress proteins will provide a better method for determining environmental contamination.

The stress proteins synthesized by an organism are a highly sensitive indicator of environmental stress, according to Welch. The amount of stress protein it produces indicates the severity of the pollution; a knowledge of which ones it makes reveals the type of stress. For example, cells make one particular stress protein, identified by its size as a sub-unit molecular weight of 32 kilodaltons, in response to heavy metals, but not in response to other stress agents. So if researchers identify this protein in an organism, they know that heavy metals are present.

Some industries could use this knowledge, Welch argues, to monitor the pollution of waterways near factories, for instance, by placing a specific test organism at a site where contamination is suspected. Laboratory workers could then analyse the organism at regular intervals to determine which stress proteins are present and in what quantities.

This application is being pursued by a newly formed Canadian company called CB Research International Corporation based in British Columbia, with an American affiliate in Long Beach, California. The company, which is the first in the field, plans to market diagnostic tools to measure stress proteins. The diagnostic kits might use antibodies to various stress proteins to determine their levels, or gene probes that detect the proteins indirectly, by targeting levels of messenger RNA, the chemical mediator between genes and proteins.

The company also plans to sell transgenic organisms that could act as custom-made monitors of specific pollutants. The transgenic organism would carry a "promoter" from a stress protein gene attached to a "reporter" gene. The promoter is a piece of DNA that lies alongside a gene and ensures its activation in the right circumstances. The idea is that a particular kind of environmental stress will activate the stress promoter and cause the cell to produce the reporter protein, which could be anything that is easy to detect. The level of the reporter protein indicates the severity of the stress.

Occupational health workers or environmental health officers could monitor the exposure of humans to pollution in a similar way, by taking regular blood samples and measuring the stress response of the white blood cells known as lymphocytes. An increase in the levels of stress proteins in lymphocytes above normal levels will indicate that a worker may have been exposed to a toxic compound. Welch, who also acts as a consultant to CB Research International, says that the monitoring is simple enough to be done as often as once a month.

Stress proteins will also play an important role in hyperthermia, the application of heat to tumours. Doctors are increasingly using heat, applied to tumours once or twice a week, in conjunction with a course of daily radiation treatment. Surface tumours are most easily heated with microwaves, but radiofrequency waves or ultrasound can be used to heat deep seated tumours.

The level of stress proteins increases after the first heat treatment and, at the same time, the tumour cells develop a temporary resistance to the damage caused by heat. This resistance is known as thermotolerance. As the stress proteins decrease again, the resistance also disappears. This link between the amounts of stress proteins in a cell and its tolerance to heat is the reason why scientists believe that stress proteins do something to protect cells from the harmful effects of the environment.

A clinician can decide when to give the second heat treatment, making sure that the temporary resistance to heat has disappeared first, by measuring the stress proteins. At Stanford, in the Department of Radiation Oncology, they are running a trial to test this use of stress proteins.

The proteins could also help doctors to determine the extent of many kinds of injury. When traumas occur in organs of the body, it may be difficult to track down the site and the extent of the damage. Scientists have recently shown that a stress response occurs in the heart and brain of people following the transient blockage of blood vessels, ischaemia, during a heart attack or stroke. According to Welch, it may be possible to diagnose the extent of damage caused by ischaemia by measuring the stress proteins released into the blood. Further studies may reveal stress proteins that are specific to a particular organ, enabling doctors to pinpoint the site of damage.

Stress proteins may also play a role in the development of artherosclerosis, the disease of the walls of the arteries that often leads to ischaemia, according to Paul Berberian from Wake Forest University in North Carolina. Fatty plaques develop on the walls of diseased arteries and increase in size to the point where they impede blood flow and stimulate clotting. Berberian has found that the cholesterol-rich cells in the plaque have increased levels of the most prominent stress protein, the 70 kilodalton protein. No one yet knows how the stress proteins contribute to the build-up of plaques, however.

Doctors may soon exploit the phenomenon of thermotolerance, or tolerance to stress, to reduce the effect of trauma during surgery or other therapies. Mary Barbe from the Medical College of Pennsylvania and her colleagues have shown that inducing cells to produce stress proteins by heating them mildly can protect the retina of rats from subsequent damage by light. William Currie and his colleagues from Dalhousie University, Nova Scotia, have demonstrated that the hearts of rats exposed to mild hyperthermia recover more quickly from ischaemia than do hearts that have not been heated. So using hyperthermia to stimulate cells to produce stress proteins might help to protect the retina during eye surgery or the heart following ischaemia.

Parasitologists are studying stress proteins as well. Parasites that cause malaria, schistosomiasis and sleeping sickness, for instance, suffer a heat shock when they invade a human. Their body temperatures rise from that in its primary host, such as a snail, to 37° C in humans. Douglas Young from the Medical Research Council's Tuberculosis Unit at Hammersmith Hospital, in London, has shown that the parasites secrete large amounts of the 70 kilodalton stress protein, and that the patient responds by making antibodies against it. The bacteria that causes leprosy, legionnaires' disease, syphilis and Lyme disease also elicit an antibody response, but against a different stress protein.

Because the stress proteins stimulate the body to produce antibodies, they could be candidates for vaccines against these pathogens. The stress proteins made by all organisms have much the same structure - they share "highly conserved" regions - so a vaccine against stress protein should protect against many diseases caused by parasites. The structural similarity of stress proteins, however, may cause problems for the human host. Repeated injections of a protein vaccine may break down the host's tolerance of self. The antibodies produced may turn around and attack host stress proteins causing an autoimmune reaction. At the moment, it is unclear who benefits from the stress proteins during infection, the pathogen or the host. But either way, manipulating the level of stress proteins during infection may reduce the severity of the infection.

The booming field of biotechnology also stands to benefit from the application of stress proteins and their promoters. Industry now produces many proteins that have important uses in medicine, such as insulin and growth hormone, from genetically engineered bacteria and yeast cultured in large fermentation vats. Various sorts of environmental insults, such as crowding, overheating and nutritional deficits cause a stress response in the yeast or bacteria that reduces the amount of the desired protein they can manufacture. CB Research International plans to develop strains of yeast and bacteria that produce unusually high levels of one or more stress proteins and so are resistant to, or tolerant of, environmental stress in the vat.

The company also has industrial outlets in mind for its pollution indicator. This consists of a promoter from a stress protein linked to a reporter protein. Company scientists will transfer the construct into the yeast or bacteria along with the gene for the protein being made for commercial purposes. Any stressful situation in the vat would activate the promoter and cause the cells to manufacture the reporter protein. By measuring the amount of the reporter protein, for example by detecting a change in the colour of the medium, technologists could receive an early warning of stress in the vat.

Biotechnologists are also exploring the opposite approach: to link the gene for a protein of commercial interest to the promoter of a stress protein. Then, as harmful conditions develop in the vat, cells will reduce their synthesis of many normal proteins but increase the production of the stress proteins. Cells will make more of the commercially important protein as well, because the gene for the protein is linked to the stress promoter. Technicians can then easily harvest the desired protein from a medium containing few other normal proteins.

Tolerance to stress can be used to advantage in agriculture too. Genetically engineered plants containing extra copies of the genes for stress proteins could grow in environments that normally plants cannot tolerate. It may be possible to produce crop plants resistant to heat, drought or salt. As the greenhouse effect continues to promote global warming, humanity will increasingly rely on plants that can grow at higher temperatures. Scientists have yet to determine which of the stress proteins can provide this protection. (Source: New Scientist, 1 April 1989)

Gene technology to spot pollution damage

As a project under the Swedish National Environment Protection Board scheme, a research group has been set up at the Lund Institute of Science and Technology to study the early identification of pollution damage by gene technology. The research objective is to improve early detection of acidification, heavy metals, dioxins and organic solvents.

The current theory is that organisms in the soil adapt to environmental changes, and studies of their germ plasm with DNA technology could therefore indicate a process of change in the environment.

The overall aim is to develop new sensitive methods to spot early stages of change in the environment that at present remain undetected. The problem of soil testing is that changes take place very slowly over decades until the tolerance level is reached. Then deterioration rate escalates rapidly to reach a "beyond repair" stage within a short period. (Source: BIO Technica Journal No. 2, 1989)

Waste water purification by fungus

Scientists at the Vienna Technical University's Institute for Biochemical Technology and Microbiology have developed a process in which the organic chlorine compounds in the waste water from bleaching cellulose pulp are broken down by white pocket fungi, which in nature utilize the lignin of wood. The resulting compounds have no effect on rivers and streams. An efficiency was reached in initial trials and a pilot plant is due to be installed at a cellulose pulp factory in late 1989. (Source: BIO Technica Journal No. 2, 1989)

In-situ soil decontamination

The FRG Bergbau Forschung GmbH with several partners works on the microbial degradation of contaminants in soils of sites where coke oven plants and gas works operated before. The objective is to develop a testing procedure in order to evaluate efficacy beforehand and input of biological reclamation of contaminated soils. The results of the laboratory tests will, after assessment of geological and hydro-geological site parameters, pave the way for the design of an efficient reclamation strategy. (Source: BIO Technica Journal, No. 2, 1989)

Biological denitrification of drinking water

Based on R&D work of the Preussag AG, a demonstration plant for the biological denitrification of drinking water has been tested at Langenfeld-Monheim. The DENIPOR process applies heterotroph aquatic micro organisms and ethanol to transform 95 to 99 per cent of the nitrates into gaseous nitrogen without changing the natural composition of water. The plant is designed for a reduction of 80 Kg/d N equivalent to 360 Kg/d $NO^3$. About 300 $m^3$/h of water are nearly completely denitrified. (Source: BIO Technica Journal No. 2, 1989)

Bacterial armoury joins fight against Alaskan spill

Scientists with the Environmental Protection Agency (EPA) in the US plan to use bacteria that consume oil in an experimental attempt to tackle the oil spill which has fouled the beaches of Alaska's Prince William Sound since the tanker, Exxon Valdez, ran aground there in March.

Jay Benforado, of the EPA, says that the bacteria are not "superbugs" genetically engineered to break down oil. They are the hardiest natural survivors of the original spill, the worst in American waters. The scientists plan to encourage the growth of a "suite of organisms", assuming that one or more strains will be able to degrade the toxic hydrocarbons in the spilled oil.

This is the first time that such biological techniques have been used to tackle large-scale oil spills. The investigators will develop rules for the study as they go.

The intention is that researchers should periodically feed bacteria living on the beaches with a fertilizer that is rich in phosphorus and nitrogen. In a separate approach, others will take samples of local bacteria to laboratories in the area around Valdez. There, they will be cultured and grown into a large biomass which will be returned to the beaches for active duty. The team will then compare the effectiveness of the two approaches.

There is little fear that the bacteria will grow uncontrollably, as their food supply can be interrupted at any time, says the EPA. The investigators will, none the less, monitor the experiment carefully for unwanted side effects such as "eutrophication", a rapid growth of algae that can occur when nutrients are unusually abundant.

Blooms can kill fish and other marine organisms by depriving them of oxygen. By-products of bacterial degradation will also be analysed. The team fears that such by-products may poison nearby plants and animals. (Source: New Scientist, 10 June 1989)

Crude oil degradation micro-organisms from ATCC

In the wake of the Exxon Valdez disaster in Alaska, the American Type Culture Collection (ATCC) has announced that it has 26 micro-organisms which have been cited in scientific publications for their ability to degrade petroleum, i.e. crude oil. The group of organisms includes six bacterial strains, six yeast strains and 14 fungi strains. Details from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852-1776, USA. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Adapting medical diagnostics

EnSys Inc., a privately held environmental biotechnology company, has just raised $4.5 million through the sale of equity to a group of venture capital funds and plans to develop rapid, easy-to-use, on-site diagnostic tests for the detection of hazardous materials.

The company's first products will be directly readable air monitoring badges to be worn by workers, a project on which it is collaborating with Assay Technology, Inc. These will be followed by test kits to detect chemicals and toxic waste in soil and water samples.

The EnSys strategy involves adapting medical diagnostic technology for environmental use. New tests, like those used to test for pregnancy at home, are designed to be used by consumers. Many are based on immunoassay, the process in which antibodies are used to detect minute quantities of substances. EnSys is developing hazardous chemical

detection systems using both immunoassay and colour change chemistry.

The advantage of the new environmental diagnostic tests is that they should be highly sensitive, yet easy to perform. As a result, expensive laboratory equipment or highly skilled technicians are not needed. Instead, the tests could be used by workers at all stages of hazardous waste monitoring or at toxic waste clean-up sites. Worker monitoring badges using this technology could be read directly by the wearer, simply by observing a colour change.

Among the substances the EnSys tests will detect are chemicals like aromatic hydrocarbons, chlorinated solvents, pesticides and heavy metals. Details from: Orlan Johnson, President, EnSys Inc., 300 Park Offices Drive, Suite 115, P.O. Box 14063, Research Triangle Park, NC 27709, USA.

Note: A report focusing on emerging clean-up technologies has just been published in the States. Copies of Cleaning up: US Waste Management Technology and Third World Development, by John Elkington and Jonathan Shopley, are available from the World Resources Institute, 1709 New York Avenue, N.W., Washington, D.C. 20006, USA. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

Genetic engineering improves ethanol yields

Through genetic engineering, SERI researchers are lowering the cost of producing fuel ethanol from cellulosic biomass feedstocks such as wheat straw and wood chips.

The key is in the production of xylose isomerase, a bacterial enzyme that increases the efficiency with which xylose, a five-carbon sugar, is fermented to ethanol. Xylose accounts for 30 to 50 per cent of the fermentable sugar in these feedstocks.

Using a genetic engineering technique that allows for the overproduction of enzymes, researchers in SERI's Biotechnology Research Branch have achieved cost-effective production of xylose isomerase.

This enzyme converts xylose to another sugar, xyulose, which can be more easily and efficiently fermented by yeast. By simultaneous fermentation and isomerization of xylose (SFIX), yeast cells continuously convert the xylulose formed into ethanol, using all the available xylose and increasing the rates and yields of ethanol production.

SERI researchers developed the process of overproduction of the enzyme xylose isomerase through genetic engineering. The gene responsible for production of the enzyme in the bacterium Escherichia coli was altered by adding a promoter gene, a segment of DNA that makes the original gene produce xylose isomerase at higher than normal rates. To allow researchers to control the timing of the overproduction, a repressor gene was also added. The repressor makes the production of the enzyme temperature dependent.

Controlling the timing of enzyme production allows researchers to fine tune the process by growing the E. coli cells and producing the enzyme in separate steps. The cells are first grown to high density at 32° C, the optimum temperature for growth. The temperature is then raised to 42° C, which induces the enzyme production. This results in an enzyme concentration equal to about 20 per cent of the total protein in the cells after two hours of induction, roughly 10 to 20 times the level normally produced by E. coli. SERI scientists believe these results can reduce the cost of ethanol production from $1.80/gal. to $1.35/gal. Contact: Dr. Stan Lastick (303) 231-7279. (Source: SERI S&T In Review, Winter 1988-89)

Microalgae consume greenhouse gases while producing fuel

Microalgae may one day trap some of the carbon dioxide responsible for the greenhouse effect and turn it into liquid fuel and other useful products. These single-cell plants, which consume 20 times more carbon dioxide per unit of land area than crop plants, are the key to such facilities.

Since 1979, SERI researchers have been developing microalgae as a biomass resource for the production of liquid fuels. Their research has resulted in methods of using microalgae to biologically trap carbon dioxide, which is produced by the burning of coal and oil and contributes to global warming (the greenhouse effect) as it accumulates in the atmosphere.

Under current plant for microalgal biomass-to-fuel technology, microalgae have the potential of using 160 billion kilograms of carbon dioxide per year for production of fuel and other products such as specialty fatty acids and natural pigments.

The use of microalgae is preferable to other carbon dioxide reduction schemes, which, some analysts predict, would greatly affect existing utility operations and increase the cost of electricity by at least 75 per cent.

The desert south-west of the USA, with abundant expanses of flat land, high solar radiation, few competing land uses, and availability of large saline aquifers, is an ideal initial location for microalgae farms. Other areas of the country and globe also have potential for such installations. Contact: Dr. Lew Brown (303) 231-1321. (Source: SERI S&T In Review, Winter 1988-89)

Biopesticides: Can they herald the promised land?

According to a recent survey chemical pesticides are going to be slowly replaced by biologicals, a collective name for biopesticides - the insect toxins, pheromones, growth regulators, etc. in the coming decades. The present world turnover of about $30-34 million is likely to grow to $8 billion within the next ten years to come. Since 1964, when DDT was banned, another 15 pesticides were banned in the US. Several dozen others have been restricted or altogether banned in subsequent years up to 1986. The cost of R&D and time taken for registration (about $1 million a year for eight years) are making any new project out of reach for many companies. Another discouraging feature is that, by the time registration is granted, a little more than half of the patent period of 15 years expires. The US Congress has recently extended the patent by five years to offset the time taken for registration. As against this biopesticides take three to four years to develop and cost a third of what it costs to develop a chemical molecule.

Again, it is the American scene where the action is, though research is going on all over the world, on non chemical pest control agents. Several biotechnology companies are in the fray, developing

new microbes. About a score of leading pesticides giants are also in the run. Sandoz has introduced Bacillus thuringiensis (BT) and has demonstrated its effectiveness as a mosquito larvicide also. Monsanto went another step ahead and developed a genetically engineered Pseudomonas fluorescens, which incorporated the insect toxin-producing gene of BT. These organisms are expected to colourize in the roots of plants and kill soil insects. Pseudomonas could not get the EPA clearance for field testing, as there is no information on the migration of the bacterium. Another biopesticide was rejected by EPA as the manufacturers, Advanced Genetic Sciences, a California company, conducted unauthorized field trials with its genetically engineered P. syringas (ice minus strain). Following this, Monsanto has dropped its application for M. fluorescens. As a follow-up, Monsanto has developed another genetically engineered strain of lactose digesting P. florescens, which turns bright blue due to a genetic market. This will make it easy to detect the bacteria if they migrated away from the root clusters of the plants where they are deposited.

According to industry sources, however, the real potential lies in toxins of fungal and viral origin. Monsanto is developing a fungal strain, alternaria cassiae, which can kill jointvetch, a weed of soybean in the US, and also have a programme of joint effort with Mycogen, a biotechnology company, which is likely to culminate in marketing the product by 1990, under the trade name "casst". Notwithstanding the scientific merits and Monsanto's interest, fungal pesticides are not much to talk about in commercial terms. The temperature and humidity in the country are said to be capable of neutralizing their effectiveness.

Chevron Chemicals' Agricultural Chemicals Division is engaged in actively evaluating a fungicide and is negotiating with biotechnology companies about setting up joint ventures. Hoechst-Roussel Agri. Vet claimed that they have a non-selective pesticide which they are hoping to register in the US, also by about 1990.

Dow Chemicals is adopting a different strategy. Dow has acquired United Agriseeds, one of the 10 top US seed companies, at a cost of $45 million to make forays into bio-pesticides, while American Cyanamid is using both in-house capabilities and collaboration. They have agreements with some companies working in the field. Ecogen, a Pennsylvania-based company, is working on a cotton bio-insecticide, with American Cyanamid. The company has already commercialized two biopesticides, "Dagger G", a biofungicide based on naturally occurring Pseudomonas fluorescens for controlling the damping of the disease of cotton seedlings, and another a collage biopesticide for the control of jointvetch weed in rice and soybeans. Ecogen is also on the lookout, according to reports from the joint venture partners, for marketing its products.

Another company to have hit the headlines and coming closest to a recent trend in the US is Igene Biotechnology, who commercialized a nematicide Clando San, derived from the shells of crabs, shellfish and shrimps. The company's chairman reportedly said that the product has a market potential of $1 million a year.

In India, the Neem Mission of Pune is popularizing Neem-derived products. A technology for the production of Neem products is available with IARI. A fungicide of plant origin "Phytoalexin" is marketed by the West Coast Rasayan. The product induces phytoalexin production in the plant body which wards off phytomycetes infection. This is almost on the same lines as the technique of immunization. The product based on Phytoalexin produced by gram plants is being marketed among the grape farmers in Maharashtra, Andhra Pradesh and Karnataka. The manufacturers cite references to say that this chemical cannot be synthesized in the laboratory.

Though several biological control agents are recognized and references to herbs with pesticidal properties are abundant in Ayurveda, and the folklore, no Indian company has attempted to develop and commercialize these products. The comparatively low cost of R&D and the possible ease with which these products can be registered should open enormous opportunities to Indian companies and scientists to develop future generation pesticides. Unlike in the West, Ayurveda and local medicines offer clues to potential candidates, which is an advantage the scientists and technocrats in India should exploit. (Extracted from Chemical Business, 20 May - 4 June 1989)

## Extraction industry applications

### Magnesite beneficiation through gene technology

The State-owned Burn Standard Co. Ltd. in Calcutta, India claims to have achieved a vital breakthrough in magnesite beneficiation by employing a genetic engineering process. Following sustained research and development efforts, the company may be able to economically utilize the large magnesite ore reserves in Salem district in Tamil Nadu. In the current fiscal year, it is setting up a pilot plant at Salem with 5-mt/d capacity. When chemical beneficiation would have cost R 200 million, or about $14 million, the company turned to genetic engineering to remove silica from magnesite ore.

The genetic engineering technique involves the isolation of various silicate micro-organisms from different magnesite ores through silica-removing strains. After extensive research, a process was developed where silica content could be reduced from 14 per cent to 2.5 per cent, and a series of reactors has been installed in a semi-pilot plant. The results show that silica contents could be reduced to 1 per cent. (Source: E & MJ [Engineering and Mining Journal], May 1989)

### Iron-oxidizing bacteria

Scientists at Dowa Kogyo (Dowa Mining Co. Ltd., Tokyo) - collaborating with researchers at the Agricultural Junior College of Akita Prefecture - have developed a host-vector system for cloning genes in iron-oxidizing bacteria. One constructed strain grows twice as fast as the wild type, and oxidizes iron at twice the rate. Iron-oxidizing bacteria are used in mining to leach metals such as copper and uranium from iron ore and to process waste hydrogen sulphide gas produced by petroleum refineries. Unfortunately, however, many of the potentially useful bacterial strains grow too slowly to be practical.

Iron-oxidizing bacteria grow in extremely acidic soil. They convert $Fe^{2+}$ to $Fe^{3+}$, using the chemical energy and carbon dioxide released during this reaction for metabolic growth. The host vector system consists of a bacterial plasmid carrying the gene for mercury resistance The scientists used this vector to clone a gene that decreases the doubling time of the host bacteria

from six to seven hours to three hours. The scientists hope to develop new "industrial-strength" bacterial strains in two to three years. Because there are still restrictions concerning the release of genetically engineered organisms into the environment, extensive safety testing will be required before the bacteria can be used in the field. (Source: Bio/Technology, Vol. 7, June 1989)

**Chemical applications**

Nitto discovers acrylamide microbe

Nitto Chemical Industry of Japan has discovered a microbe it claims is capable of improving biological production of acrylamide. The microbe will allow the company to increase acrylamide production from 6,000 ton/year to 10,000 ton/year without having to alter its facilities.

According to the Japanese firm, this microbial process is superior to the sulphuric acid and copper catalyst processes now in use. "It is capable of producing acrylamide at lower cost and calls for only limited space for installation of process units", says Nitto.

The new organism will be scaled up for commercial production during this year. Nitto intends using the technology overseas both by itself and through joint ventures. (Source: European Chemical News, 24 July 1989)

**Industrial microbiology**

SERI enzyme work increases industry profits

SERI researchers are at work lowering the expense of industrial enzyme use by producing enzymes that remain active longer under the harsh conditions encountered in industrial reactions.

Today, industry commonly uses enzymes to help break down starch in the production of alcoholic beverages, to degrade cellulose in clarifying fruit juices and modifying fruit and vegetable products, and as protein- and lipid-degrading agents in detergents and meat tenderizers.

Using special chemical reagents, SERI researchers are modifying enzymes by reticulation - the formation of a network of covalent chemical bonds between different portions of the folded peptide chains that make up the molecules of enzymes and other proteins.

The man-made supplementary bonds created by this type of crosslinking produce enzymes with greater physical stability than occurs naturally.

One example of such modification is the enzyme β-glucosidase, which is produced by the fungus Aspergillus niger and used to supplement the conversion of cellulose to glucose for fermentation to fuel alcohol. During the past year, researchers in SERI's Applied Biological Sciences Section of the Biotechnology Research Branch have developed crosslinking techniques that increase the life time of this enzyme more than 200 fold at the elevated temperature.

Through such efforts in enzyme technology, SERI is helping clients enhance their industrial processes and achieve greater profitability.

Contact: Dr. Karel Grohmann (303) 231 7252. (Source: SERI S&T In Review, Winter 1988 89)

Biotechnology headphones

Sony's MDR-R10 King is the world's first headphones produced using bacteria to make the diaphragm that vibrates to produce sound. In most headphones, the diaphragm is made of compressed paper. Sony, with Ajinomoto and Research Institute for Polymers & Textiles, uses Acetobacter aceti, a short, rod-shaped bacterium, to make the diaphragm. It feeds the bacteria with a solution of sugar saccharides to produce threads of cellulose, under 40 nm diameter each. After two days, the threads mesh into a web 2 mm thick. Sony dries the web, compresses it into a sheet 20 microns thick and shapes it into a miniature diaphragm that is ten times as rigid as paper. (Extracted from New Scientist, 25 March 1989)

**Bio-hazards**

Assessment of genetic hazards

The GENHAZ procedure for assessing the hazards that would be posed by releasing genetically engineered organisms into the environment has been developed under the sponsorship of the UK Royal Commission on Environmental Pollution. GENHAZ is based on ICI's HAZOP procedure for hazard analysis, which requires flow diagrams of various aspects of a process, and poses questions about various stages in the process. GENHAZ poses questions about each step in the release of an organism. GENHAZ will be tested in evaluating the release of genetically altered potatoes at the John Innes Institute of the Agricultural & Food Research Council. Commission member C. Suckling says GENHAZ will point out even remote possibilities. J. Beringer of the University of Bristol says that GENHAZ asks questions in a structured way that is novel to genetic engineering. K. O'Connor of the US Office of Technology Assessment says there is no comparable hazard assessment procedure in the US. (Extracted from New Scientist, 27 May 1989)

## E. PATENTS AND INTELLECTUAL PROPERTY ISSUES

EPO rejects patent application for transgenic mice

The European Patent Office in Munich has rejected an application filed by Harvard University in Massachusetts for so-called "oncomice" - transgenic mice with an activated cancer gene from humans that produces tumours in the animals. The US Patent Office granted a patent covering such oncomice to the university more than a year ago.

The inventors of the oncomice are Philip Leder from Harvard Medical School and his former collaborator, Timothy Stewart, now working for the Genentech company in San Francisco. Researchers can use the oncomice as experimental models for certain types of cancers that occur in humans.

The first commercial product based on the American patent is a strain of oncomice that carries a human oncogene (cancer gene) called ras). Du Pont, an American pharmaceuticals company, produces the mice under licence. The animals carry ras and a sequence of genetic material which ensures that ras is "switched on". Most of the transgenic animals develop a type of breast cancer within a couple of months.

Christian Gugerell, the chief examiner at the European Patent Office, said that there were two reasons why the office decided on 27 June to reject

the patent. First, Article 53(b) of the European Patent Convention excludes animal varieties from patent protection. The office interprets one of the 17 claims of the application as inherently concerning animals. Secondly, the application covered any transgenic mammal.

The US Patent Office took a different view, following a US Supreme Court ruling in 1980 that "everything under the sun" could be patented if it included some sort of invention. Harvard wanted the European office to grant a patent on the microbiological process used to produce oncomice, but its application included the claim for the animals themselves. Under the terms of the European Patent Convention, patents referring to a process also cover the products of that process. Harvard has two months to appeal against the decision. (Source: New Scientist, 8 July 1989)

Draft directive on patentability of biotechnological inventions in the European Economic Community

The European Commission has sent to the Council a formal proposal for a directive on patentability of biotechnological inventions, with the aim of ensuring that protection in this field is available on similar conditions in all member States of the European Economic Community.

According to the draft, the following will be patentable:

- Micro-organisms (i.e. all microbiological entities capable of replication)
- Biological classifications other than plant or animal varieties
- Parts of plant and animal varieties other than propogating material protectable under Plant Variety Protection law
- Plants and plant material, unless produced by non-patentable use of a previously known biotechnological process
- Uses of plant or animal varieties, and process for the production thereof
- Microbiological processes (i.e. processes carried out with the use of, performed on, or resulting in a micro-organism
- A process in which human intervention consists of more than merely selecting an available biological material and allowing it to perform an inherent biological function under natural conditions.

The subject matter of an invention, including a mixture, which forms an unseparated part of a pre-existing material, will not be considered unpatentable for the reason only that it forms part of the natural material.

Much consideration has been given to the scope of protection, the circumstances in which use is regarded as experimental, rights to protect propagation of self replicable products, the extension of rights to the product of a process and to subsequent generations obtained by it, and the extension of protection for a product with particular genetic information to any product incorporating that information, where this information is an essential characteristic of the invention and is essential for its industrial utility.

The most important new proposal is to reverse the burden of proof in an action for infringement, where a process for making a product has been patented, and that product is produced by a party other than the patentee, and an organism for carrying out the process has been deposited and a sample given to a third party. In these circumstances, in the absence of proof to the contrary, the product will be deemed to have been obtained by the patentable process. This provision would give much greater protection to the patentee where a new biotechnological process for manufacture of a known product has been patented, and the micro-organism has been deposited. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

Patenting Life Forms in Europe - a new publication of the ICDA Seeds Campaign

The patenting of life forms has grown into a highly controversial issue, now that the new biotechnology products are starting to reach the marketplace. The EEC is about to take decision on a draft Directive which would make virtually all forms of life subject to exclusive monopoly control in all EEC member States. The US Patent Office already granted industrial patents on plants and animals, while developing countries are under increasing pressure to do the same.

Most of the decision-making on patenting life forms is being done within board rooms and lawyers' offices, while the impact of life patents will be felt by all of us. Deep concern has already been expressed by breeders' and farmers' unions, consumers and church-based organizations, and by environment and third world groups. The heart of the question is whether genetic resources should be privately owned, and whether society should grant monopoly control over them to a handful of large transnational corporations that are already controlling the development of the new biotechnologies.

In order to provide a platform for the public interest community to openly voice its concerns on the impact of life patents, the ICDA Seeds Campaign and GRAEL convened an international conference at the European Parliament on the patenting of life forms (February 1989). The conference was attended by almost 200 people from many different sectors, including NGOs, private industry, government officials and public research institutions. ICDA has now published Patenting Life Forms in Europe, in which the full texts of all interventions - 18 in total - are reproduced.

Patenting Life Forms in Europe brings together, for the first time, a whole range of data, views and opinions on the impact of life patents presented by policy makers and public interest groups. What do patents mean for the biotechnology industry, for public research, for farmers and for breeders? What are the ethical and religious concerns and how will they affect the position of developing countries? Written in understandable language, Patenting Life Forms in Europe intends to broaden the discussion on life patents and present accessible information and views to facilitate the work of policy makers and NGOs. The book has 80 pages, tables/graphs/ illustrations and costs US$30 for individuals and others and US$12 for NGOs. Available from ICDA Seeds Campaign, Apartado 23398, E-08080 Barcelona, Spain.

European Patent Office narrows definition of "medical treatment"

In a decision of the European Patent Office's Technical Board of Appeal (Case T385 86, 25 September 1988) the definition of "medical treatment" for the purposes of patentability was considerably narrowed. The application was for a method for non-invasive determination of chemical and/or physical conditions inside a living animal or human body using magnetic resonance. The Examiner rejected the application as being for a method of diagnosis under EPC Article 3 52(4), and hence unpatentable. It was held that Article 52(4) must be construed narrowly, and excludes from patentability only methods whose results directly enable a decision as to a particular course of treatment. Methods providing interim results are not diagnostic methods for this purpose, even if they can be used in making a diagnosis. It was further held that a method of data gathering per se does not amount to a method of diagnosis, since this requires further steps of comparison with normal values, identification of abnormalities, and a decision as to whether these abnormalities result from a clinical condition. All of these steps are required in order to constitute a diagnostic method. A method which involves interaction with the human or animal body can be industrially applied if it can be used by a technician who does not have specialist medical knowledge or skills. In order to fall within Article 52(4), a diagnostic method to be practiced on the human or animal body presupposes a symptom which is directly discernable on the body itself. It was held that the claims were not for a diagnostic method as defined by Article 52(4), but were for a technical method of measurement which might be useful for diagnostic purposes, as well as for other purposes.

It is thought that this decision may open the way for patentability of non-invasive methods of diagnosis. This, in conjunction with the decision regarding patentability of transgenic plants, widens the scope of protection available in Europe for biotechnological inventions. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

Animal patents

Animal rights, the release of genetically engineered organisms into the environment, and the effects of large agribusiness companies on the farming industry are the real issues behind the debate over the patenting of animals, according to a report* released by the US Congress's Office of Technology Assessment (OTA). Existing regulations can be adapted to address most of the practical considerations of animal patenting, such as whether farmers should pay royalty fees for breeding patented livestock. But the ethical question of whether or not transgenic animals should be subject to patents is a question that may need further clarification, OTA says.

Congress had heard both sides of the debate since the patent office declared in early 1987 that it would not reject patent applications on the sole basis that the invention for which the patent was being sought was an animal. The granting of the first patent for a higher life form last year to Harvard University, for a transgenic mouse containing human oncogenes - heated up the exchange between patent proponents and those who asserted that animal patenting would lead to animal suffering and higher costs for farmers.

But attempts in the past two years to pass legislation to halt the patenting of additional animals until the ramifications could be worked out - and specifically to exempt researchers and farmers from royalty payment requirements - have floundered. The Animal Legal Defense Fund has also lost the first round in a court battle to overturn the patent office's decision to patent animals.

In the meantime, 44 patent applications covering animals have piled up at the patent office. Other companies are not waiting for patent protection to develop profit-making genetically engineered animals. Integrated Genetics has formed a collaboration with Tufts University to develop herds of cows capable of excreting human proteins in their milk, and the company Transgenic Sciences has just been formed, and intends to develop animal breeds which produce valuable pharmaceuticals. (Source: Nature, Vol. 338, 30 March 1989)

* New Developments in Biotechnology Patenting Life, Office of Technology Assessment, Washington DC, 1989

## F. BIO-INFORMATICS

"Alternative" pesticides spawned by biotechnology could grow to $8 billion market by 2000

An emerging world market for "alternative" microbiological and biochemical pesticides could be worth $8 billion by the end of the century, according to a new 350-page study from Frost & Sullivan entitled The Impact of Biotechnology on Pesticides in the US (A2150).

"Many of the genetically engineered products coming out of the laboratory over the next few years will need to sustain three to five years or longer of large-scale field testing and clearance before market release", the report says. "For those who can survive this passage, the rewards will be the ability to participate in a market that could reach $1-2 billion within 10 to 15 years in the US and $6-8 billion worldwide".

The report also advises both large and small companies to develop strong expertise and proprietary processes in targeted key technologies; select "appropriate problems to solve within high value markets"; consider strategic relationships for research, production, marketing, distribution and financing; and think in terms of large-scale production.

Alternative pesticides will be developed from several core technologies. Recombinant DNA techniques will create micro organisms to be used as pesticides, disease suppressants and chemical detoxicants, as well as making plants themselves more intrinsically pest resistant.

Bioprocessing will produce large quantities of alternative pest controls efficiently and at lower costs. Detection and measurement systems will be developed to monitor plants and their environment for pathogens and selected chemicals. Ultimately, advances in basic cell biology will yield greater knowledge of insect and plant physiology leading to new methods of pest control. Alternative insecticides and herbicides will include bacteria, viruses, fungi, other micro organisms and

regulatory messenger control chemicals. Details of the report, priced at $2,900.00, from: Customer Service, Frost & Sullivan Ltd., Sullivan House, 4 Grosvenor Gardens, London SW1W 0DH. In the States, Frost & Sullivan Inc., are at 106 Fulton Street, New York, NY 10038. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Biopesticides report

A new report on biopesticides from CPL Scientific Information Services predicts that protecting crops by non-chemical means is likely to become far more widely practised in the near future. Although alternative means of pest control, including biopesticides, have thus far failed to fulfil earlier predictions that they would take a significant share of the world agrochemicals market, they are now poised to pose a challenge to agricultural chemicals.

In the current climate of anxiety about contaminated food and water pollution, consumers are suspicious of chemicals and would like to see their use reduced. In the past biopesticides did not represent a feasible alternative to "conventional" chemicals, as they were frequently unreliable and expensive. Farmers attempting to grow "organic", non-chemical food found it difficult to obtain the same quantity and apparent quality of produce grown with chemicals.

Improved biopesticides now represent a real alternative to chemical crop protection. Genetic engineering and other techniques have been used to make plants resistant to insects and to make micro-organisms like bacteria, fungi and viruses take on different properties to enhance their potency as biopesticides.

Around 50 companies are taking an active interest in biopesticides including established producers like Abbott Laboratories and Sandoz, major oil and chemical companies like Shell, Monsanto and ICI, as well as new, dedicated biotechnology companies like Ecogen and Mycogen. New products coming to the market include biopesticides for the control of potato beetle, specialty products for insect control in vegetables and many others. Although the total market is no more than $20-$30 million at present and R&D expenditures exceed product sales, new technology and the new products will make the market grow.

A newly published report from CPL Scientific Information Services reviews the current status and future prospects of biopesticides in considerable detail. BIOPesticides: Markets, Technology, Registration & Companies 100,000 words in two volumes is available for 1,500 pounds sterling or $2,500 from: CPL Scientific Limited, Science House, Winchcombe Road, Newbury RG14 5QX, UK. Tel: 0635-524064, Fax: 0635 529322. (Source: Australian Journal of Biotechnology, Vol. 3, No. 2, April 1989)

Five Year Research Plan 1988 1992: Biofuels: Renewable Fuels for the Future

This publication outlines the research plan of the US Department of Energy's Biofuels and Municipal Waste Technology Program. The Program's research focuses on five pathways for producing liquid and gaseous fuels (biofuels) from many types of plant materials and from certain waste products such as municipal wastes. The fuel pathways use new or currently available feedstocks and lead to (1) alcohol fuels, (2) biocrude derived gasoline, (3) plant-oil-derived diesel fuel, (4) biogas, and (5) syngas. These renewable fuels provide alternatives to petroleum, natural gas and other fossil fuels. Background, current status and future research required are presented for each fuel pathway, as are technical performance and cost goals. The publication is available from the National Technical Information Service, US Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161.

Handbooks of Biomass Downdraft Gasifier Engine Systems by Thomas B. Reed and Agua Das

Recent concern about cost and availability of alternative fuels has revived interest in generating power from biomass fuels, a technology that has existed for decades but without the benefit of many written reference materials. This handbook is intended as a guide to the design, testing, operation and manufacture of small-scale (less than 250 kW) gasifiers though much of the information is applicable to all levels of biomass gasification. Written by two experts with more than 10 years of hands-on experience, the handbook should be useful to engineers and others needing practical information about the design, construction and operation of gasifiers. In addition to many illustrations, it contains an extensive list of references and sources of additional information. Like most documents produced by the Solar Technical Information Program, the book is also available from the Superintendent of Documents. The publication is available from the National Technical Information Service, US Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161.

The Laws of Life: Another Development and the New Biotechnologies by Cary Fowler, Eva Lachkovics, Pat Mooney and Hope Shand

According to this study, biotechnology offers the poor of the world both Einstein and Frankenstein, serving as a bright beacon to a better world, but also threatening to consume the third world and radically uproot agriculture and transform human health care. The study outlines more than 30 recommendations aimed at national Governments, the UN system, the private sector and citizen groups and religious organizations. (Co-published by the Dag Hammarskjöld Foundation and the Rural Advancement Fund International) (Source: Development Forum, May/June 1989)

Release of genetically engineered organisms

The DECHEMA Working Party on "Safety in Biotechnology" has recently issued a report entitled Considerations on release of gene technologically engineered micro-organisms into the environment. The authors are K. H. Domsch et al. The study points out the principles to be observed by a manufacturer as a precaution against risks in the case of a deliberate release of genetically altered micro-organisms or viruses. Possible hazard potentials are deduced step by step from ecological and genetic facts, and are substantiated and correlated with considerations concerning the likelihood of their occurrence. References are given to test procedures and suitable methods for genetic and ecological safeguards. The study indicates the questions likely to be answered in the course of a registration procedure and the areas in which research activity is urgently required.

The paper has 48 references and should be useful reading to those concerned with this area.

The article appeared in FEMS Microbiology Ecology 53 (1988) 201-272 published by Elsevier. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

Australian and New Zealand Biotechnology Directory

The new Directory which was released at the Eighth Australian Biotechnology Conference in Sydney on 6 February 1989 has received wide acclaim as being a publication of very high quality and accuracy.

Mr. Barry Jones, MHR, launched the Directory when he was opening the Conference and was presented with a complimentary copy. The Directory was on sale during the course of the Conference and copies can be obtained from the publishers, Australian Industrial Publishers Property Ltd., P.O. Box 8, Cowandilla, SA 5033. (Tel: (08) 234 0022; Fax (08) 234 0058).

This joint production with the Australian Biotechnology Association (ABA) has been very successful and the ABA is particularly grateful to those government departments who sponsored the ABA for the preparation of the data base. A second edition is expected at the beginning of 1990. Companies and organizations who wish to be included in the new edition and who were not in the first edition should apply immediately to the Secretary, Australian Biotechnology Association, P.O. Box 303, Clayton, Vic. 3168 for forms. Corrections and changes of address for those who have entries in the Directory should also be sent as soon as possible to the Secretary, ABA. (Source: ABA Bulletin, Vol. 4, No. 2, April 1989)

Food Biotechnology

The seventh in the Cambridge studies in biotechnology covers Food Biotechnology. "Despite its primitive base technology and lack of understanding of existing underlying science", the authors say, "the food manufacturing industry has shown a remarkably robust appetite for biotechnology". The core of the book describes in detail the development of two products, high fructose corn syrup and "Quorn", the mycoprotein which is now at the heart of a growing number of food products you can find in the supermarket. Details from: Cambridge University Press, The Pitt Building, Trumpington Street, Cambridge CB2 1RP. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Biotechnology Sourcebook

A new 362-page report from SEAI Technical Publications, Biotechnology Sourcebook, gives an inside view of the biotechnology programmes of 280 corporations, from AC Biotechnics to Zymo Genetics. The two authors, Shawn Lynn Linam and M. Todd Jarvis, are based at Mississipi State University. Details of the publication, priced at $85.00, from: SEAI Technical Publications, P.O. Box 590, Madison, GA 30650, USA. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Biotechnology publications from the OECD

The fifth in the series of biotechnology publications produced by the Organization for Economic Co operation and Development (OECD) has just been launched. Among other things, Biotechnology: Economic and Wider Impacts (1989, 11.5 pounds sterling) looks at biotechnology's implications for the quality of life, the policies of industrial firms in this area, the time scales for diffusion of biotechnologies through the economy and the prospective impacts on employment.

Other publications in the series are: Biotechnology and the Changing Role of Government (1988, 11.00 pounds sterling), Recombinant DNA Safety Considerations (1986, 6.00 pounds sterling), Biotechnology and Patent Production (1985, 8.00 pounds sterling) and Biotechnology: International Trends and Perspectives (1982, 5.50 pounds sterling). Details from: OECD Publications, 2 rue Andre-Pascal, 75775 Paris CEDEX 16, France. Also available from HMSO. (Source: Biotechnology Bulletin, Vol. 8, No. 5, June 1989)

Bibliography for bispecific monoclonal antibodies

Polycell Inc., a subsidiary of Quest Biotechnology Inc., announced that it has compiled a bibliography which references articles on bispecific monoclonal antibodies for research and clinical applications including both diagnosis and therapy. The bibliography and copies of selected articles are available upon request by contacting Dr. Werner H. Wahl, Vice President, Science and Technology. (Source: Company News Release, 22 May 1989)

Directory of Biotechnology Information Resources

Directory of Biotechnology Information Resources (DBIR), a data base comprising international sources of publicly available information on biotechnology (National Library of Medicine, Circle 304), is available now on the library's Medlars data base system. DBIR, developed by the American Type Culture Collection under contract to NLM, includes listings on computerized data bases, networks, bulletin boards, and data base vendors; molecular biology computer resources; culture collections and repositories; biotechnology centres; nomenclature committees; and biotechnology publications. (Reprinted with permission from Chemical Engineering News, 17 April 1989. Copyright 1989 American Chemical Society)

Computational Molecular Biology

A new book by Arthur Lesk of the European Molecular Biology Laboratory covers the field of computing with protein and nucleic acid sequences. It describes what data are available, what calculations can be performed, sources of data and of software, and the intelligent interpretation of results in scientific applications. Computational Molecular Biology: Sources and Methods for Sequence Analysis was written in response to an initiative from a CODATA Task Group, to bring together information of importance to scientists working on sequence analysis in molecular biology. Details of the book, price 25.00 pounds sterling, from: Oxford University Press, Walton Street, Oxford, OX2 6DP, UK. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

Biosearch Ireland

Irish Biotech News is available free on request from BioResearch Ireland. Founded in 1987, BioResearch Ireland is a biotechnology contract organization which links Irish universities and research institutes. Its research centres operate in five Irish universities and carry out research in diagnostics and biotechnology

applications in food, agriculture, veterinary science, pharmaceuticals, and cell and tissue culture.

Technical services include the production of monoclonal antibodies, diagnostic tests for fish diseases, amino acid analysis, toxicology testing, micro-encapsulation of novel drugs and protein sequencing. Details from: Mary McCarthy, BioResearch Ireland, EOLAS, Glasnevin, Dublin 9, Ireland. (Source: Biotechnology Bulletin, Vol. 8, No. 4, May 1989)

Protein Engineering Database Group

A deeper understanding of the relationships between protein and nucleic acid sequences and structures should follow from work now being done by the Protein Engineering Club Database Group. The aim is to link structure and sequence data in a single data base. The project is based at Birkbeck College, London, and at the University of Leeds, and is backed by the Science and Engineering Research Council. Among the companies involved are Glaxo, Celltech, ICI and Sturge/RTZ. Eventually, it should be possible to deduce the function of a gene simply by examining its nucleotide sequence. Details from: Ian Robertson, British Technology Group, Electronics and Information Technology Division, 101 Newington Causeway, London SE1 6BU, UK. (Source: Biotechnology Bulletin, Vol. 8, No. 3, April 1989)

New listing of ATCC recombinant DNA materials

The American Type Culture Collection (ATCC) has published a 128-page listing of cloning vectors, hosts, cloned inserts and libraries. The book does not contain oncogenes and human libraries and clones, which are available from the ATCC as part of the ATCC/NIH Repository of Human DNA Probes and Libraries. The ATCC Recombinant DNA Materials Listing is free to US customers and sent to foreign customers for a modest shipping and handling charge. Details from: ATCC, 12301 Parklawn Drive, Rockville, MD 20852, USA. (Source: Biotechnology Bulletin, Vol. 8, No. 3. April 1989)

## G. MEETINGS

1990

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| 9-11 January | Swansea, UK. SGM Symposium: Gene Transfer in the Natural Environment. Further information from The Meetings Assistant, SGM, Harvest House, 62 London Road, Reading RG1 5AS, UK |
| 16 January | London, UK. Foaming Phenomena in Bioprocessing: Opportunity or Liability? Further information from The Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 16-18 January | Fort Lauderdale, USA. Sixth International Symposium on Separation Science and Biotechnology. Further information from Mrs. Janet Cunningham, Barr Enterprises, P.O. Box 279, Walkersville, MD 21793, USA |
| 21-26 January | Miami, USA. Miami Bio/Technology Winter Symposia. Further information from Miami Bio/Technology Winter Symposia, P.O. Box 016129, Miami, FL 33101, USA |
| 1 February | London, UK. Management of biotech opportunities - a new realism for the process industries. Further information from The Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 12-15 February | Palmerston North, New Zealand. Fermentation Technologies: Industrial Applications. Further information from The Conference Director, Biotechnology Department, Massey University, Palmerston North, New Zealand |
| 14-16 February | Hobart, Australia. ANZAAS Congress. Further information from The Organizing Secretary, 1990 ANZAAS Congress, University of Tasmania, P.O. Box 252C, Hobart, Tasmania, Australia |
| 5-7 March | Stuttgart, FRG. Second International Symposium on Biochemical Engineering. Further information from Dr. W. Waldraff, ZSP, Bioverfahrenstechnik, Ko-ordinationsstelle der Universität Stuttgart, Pfaffenwaldring 9, D-7000, Stuttgart 80, FRG |
| 26-29 March | London, UK. Chemicals and Biosensors. Further information from Ms. L. Hart, Royal Society of Chemistry, Burlington House, Piccadilly, London W1V OBN, UK |
| 26-29 March | Cambridge, UK. Stability of Proteins: Theory and Practice. Further information from Prof. F. Franks, Biopreservation Division, Pafra Ltd., 150 Cambridge Science Park, Cambridge CB4 4GC, UK |
| 29 March | London, UK. Strategic use of technology - a vital issue for the 1990s. Further information from The Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 1-5 April | Delphi, Greece. EMBO/FEBS Workshop on the Structure and Function of Eucaryotic RNP. Further information from Dr. A. Gulalis, National Hellenic Research Foundation, Biological Research Centre, 48 Vassileos Constantinou Ave., Athens 11635, Greece |
| 3-4 April | Swansea, UK. Advances in Separation Processes. Further information from Dr. R.K. Sinnott, Chemical Engineering Dept., University College Swansea, Swansea SA2 8PP, UK |

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| 3-5 April | Cambridge, UK. Opportunities in Biotransformations. Further information from The Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 18-20 April | Orlando, Florida, USA. First International Conference on Human Antibodies and Hybridomas. Further information from S.L. Patterson, Butterworths, 80 Montvale Ave., Stoneham, MA 02180, USA |
| 22-25 April | Kyungju and Seoul, Korea. Asia-Pacific Biochemical Engineering Conference '90. Further information from Prof. P. Greerfield, Department of Chemical Engineering, University of Queensland, St. Lucia Queensland 4067, Australia |
| 13-18 May | Anaheim, California, USA. Ninetieth Annual Meeting of the American Society of Microbiology. Further information from R. A. Bray, American Society of Microbiology, 1913 I Street NW, Washington, DC 20006, USA |
| 20-25 May | Boston, USA. International Symposium on Liquid Chromatography. Further information from Ms. Shirley Schlessinger, 400 E. Randolph Street, Suite 1015, Chicago, IL 60601, USA |
| 22-25 May | Dijon, France. Bio-Chromatography and Molecular Affinity. Further information from Le Secretariat, Groupe Francais de Bio-Chromatographie, Unite d'Immuno-Allergie, Institut Pasteur, 28 rue du Docteur Roux, F-75724 Paris Cedex 15, France |
| 5-7 June | London, UK. Pest Management in Rice. Further information from The Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 13 16 June | Philadelphia, USA. Third International Conference on Molecular Biology and Pathology of Matrix. Further information from Dr. Darwin J. Prockop, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA |
| 25-28 June | Calgary, Alberta, Canada. Conference on Molecular and Cellular Mechanisms of Alcohol and Anaesthetics. Further information from The Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA |
| 25-29 June | Amsterdam, The Netherlands. Amsterdam Biotechnology '90 Exhibition to be held in conjunction with the Seventh Congress of the International Association for Plant Tissue Culture. Further information from RAI International Exhibition and Congress Centre, Europaplein, 1078 GZ Amsterdam, The Netherlands |
| 2-4 July | Cambridge, UK. Advances in the Chemistry of Fungicides and Herbicides. Further information from The Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 8-13 July | Copenhagen, Denmark. Fifth European Congress on Biotechnology - Biotechnology from Agriculture to Industry. Further information from ECB-5, Spadille Congress Service, Sommervej 3, DK-3100 Hornbaek, Denmark |
| 12 18 August | Strasbourg, France. Sixth International Symposium on Genetics of Industrial Micro-organisms. Further information from The Secretariat, GIM 90/SFM, 28 rue du Docteur Roux, F-75724 Paris Cedex 15, France |
| 26-31 August | Berlin, FRG. Eighth International Congress of Virology. Further information from H. Zeichhardt, Congress Secretary, Institute for Clinical and Experimental Virology, Free University of Berlin, Hindenburgdamm 27, D-1000 Berlin 45, FRG |
| 26-31 August | Vienna, Austria. Euroanalysis VII. Further information from The Secretariat, Interconvention, P.O. Box 80, Vienna A-1107, Austria |
| 28 August - 3 September | Regensburg, FRG. Fourth International Mycological Congress. Further information from Prof. Dr. Andreas Bresinsky, Botanisches Institut der Universität, D-8400 Regensburg, FRG |
| September | Beijing, China. Biotech Expo '90 Third Round of the International Exposition and Symposium on Biotechnology and Life Sciences. Further information from Commedia CICS Ltd., 22/F Sing Po Building, 101 King's Road, North Point, Hong Kong |
| 9-14 September | Interlaken, Switzerland. Fifth International Symposium Molecular Genetics of Plant-Microbe Interactions. Further information from Dr. Hauke Hennecke, Mikrobiologisches Institut, ETH-Zentrum, CH 8092 Zurich, Switzerland |

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| 11-13 September | University of Reading, UK. Second International Conference on Separations for Biotechnology. Further information from the Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 16-22 September | Osaka, Japan. IUMS Congress - Bacteriology and Mycology. Further information from Dr. Yoshifumi Takeda, Secretary General, IUMS Congress, c/o The Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan |
| 24-27 September | Gold Coast, Australia. Ninth Australian Biotechnology Conference. Further information from Prof. P. Greenfield, Department of Chemical Engineering, University of Queensland, St. Lucia, Queensland 4067, Australia |
| 28-31 October | San Francisco, USA. Anabiotec '90. Third International Symposium on Analytical Methods in Biotechnology. Further information from Ms. Shirley Schlessinger, Anabiotec '90, 400 E. Randolph Street, Chicago, IL 60601, USA |
| Date unknown | New Delhi, India. Biotek India '90. Further information from Ms. Anu Kapor, Convex, 14-F Basant Lok, Vasant Vihar, New Delhi 110057, India |
| 1991 | |
| 7-11 April | Southampton, UK. Neurotox '91. Further information from The Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK |
| 10-13 April | Boston, USA. International Symposium on Pharmaceutical and Biomedical Analysis. Further information from Ms. Shirley Schlessinger, 400 E. Randolph Street, Suite 1015, Chicago, IL 60601, USA |
| 9-15 June | Frankfurt am Main, FRG. ACHEMA '91. Further information from DECHEMA, P.O. Box 970146, D 6000 Frankfurt am Main 97, FRG |
| 15-17 July | Rothamsted Experimental Station, Harpenden, Herts., UK. Resistance '91 - Achievements and developments in combating pesticide resistance. Further information from Dr. B. Khambay, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, UK |
| August | Jerusalem, Israel. Fifteenth International Congress of Biochemistry. Further information from Dr. N. de Groot, Department for Biological Chemistry, Hebrew University, Jerusalem 91904, Israel |
| 24-27 September | Leeds, UK. Biotech UK. Further information from Biotech UK Information, c/o Prof. J. D. Bu'Lock, Manchester University, Manchester M13 9PL, UK |
| 1993 | |
| | Italy. Sixth European Congress on Biotechnology organized by Italian Member Societies |

## SPECIAL ARTICLE

### Bacterial Leaching: A Potential for Developing Countries

Rohini Acharya*

Paper prepared for the Genetic Engineering and Biotechnology Monitor United Nations Industrial Development Organisation

IFIAS-Maastricht

### Bacterial leaching: a potential for developing countries

**Abstract**

Although bacterial leaching or the recovery of metals from ores through the use of bacteria is not a new technology, the optimization of the process is still in its development stages. Bacterial leaching occurs when certain bacteria which inhabit the relatively acidic waters of mines interact with the ore to release an effluent from which the metal content can be recovered quite easily. The process has occurred in nature for hundreds of years. However, scientists have only recently started to examine means by which this process may be optimized, reducing thereby some of the highly capital intensive mining and extraction procedures in conventional mining.

Bacterial leaching offers many advantages over conventional technology. It is a natural process which already occurs in mine dumps, cutting out the costs of mining the ore and bringing it up to the surface. Furthermore, it has been discovered that bacteria can leach ores with a grading of as low as 0.01 per cent, which increases substantially the

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* The author is currently working at the International Federation of Institutes for Advanced Study (IFIAS) in Maastricht, the Netherlands as a Programme Officer in the Biotechnology Programme. This paper is partly based on a previous report funded by the GAIA Institute for the Study of Natural Resources also based in Maastricht, which is soon to appear as part of the IFIAS Biopolicy Research Series. I would like to express my thanks to Dr. Alyson Warhurst from SPRU at the University of Sussex and IFIAS for their help in the research and writing of this report.

amount of metal which can be recovered. Because the use of this technology can lead to the production of metal in a relatively pure form at the mine site, it bypasses the use of smelters, thereby reducing damage to the environment. This method is also advantageous to developing countries which often do not possess the capital to construct smelters and have to ship the ore overseas for refining, thereby losing the advantage of value added.

This study examines the use of bacterial leaching in the recovery of five metals, gold, silver, copper, cobalt and manganese and the potential this technology holds for developing countries. For this we also examine the economics of bacterial leaching in comparison with conventional processing techniques used in mining. The goal of the study is to demonstrate the viability of bacterial leaching as an alternative to conventional technologies especially to developing countries.

## 1. Introduction

Bacterial leaching, or the action of bacteria on mine ores to release an effluent containing metal is a natural process which has occurred for centuries. Bacteria which live in the relatively acidic waters of a mine are able to dissolve normally insoluble sulphide ores to release various metals in an effluent form from which the metal can then be recovered relatively easily. Similarly, scientists have recently discovered that bacterial leaching can be used for refractory gold, the extraction of which is not normally possible. Refractory gold is freed from the ore through bacterial leaching and the metal can then be recovered through normal leaching procedures. Bacterial leaching can be applied for the recovery of other metals as well in this way.

Although the technology is relatively old and would occur naturally regardless of human involvement, recent research in this field has developed quite rapidly. Present research can be distinguished from past applications because it focuses on the optimization process. This includes the use of genetically engineered bacteria to accelerate leaching or altering pH levels in the mining environment to improve recovery rates.

The development potential for bacterial leaching in mining throughout the world is enormous. Lower and less sophisticated capital requirements as well as an increase in the recovery of metal from ore offer bright prospects for the future.

Recent years have also seen an acceleration in the applications of bacterial leaching. The impetus for this change has been provided by a number of new developments which occurred over the last decade. Mining companies have had to cut costs and adhere to stricter environmental regulations in developed countries. The collapse of most base metal prices in the late 1970's and early 1980's also resulted in a worldwide restructuring programme as mining companies were forced to lower costs of production and this period witnessed large mine closures in many developed countries. The development of advanced material technology has provided a considerable threat to conventional metals as well. These are some of the more important changes which have forced the usually conventional mining industry to consider bacterial leaching as a viable option, resulting in a substantial increase in research and applications of biotechnology in the mining industry.

For the developing countries, the possibilities for using bacterial leaching are excellent. In the past, less sophisticated mining technology in these countries has resulted in higher grade ores being rejected. Thus the dumps of "waste" which have been created at mining sites contain ore of a much higher grade than presently being mined in these countries. Biotechnology can be used to recover metal from these dumps without the extra costs of mining the ore, enabling these countries to develop their mineral resources more thoroughly.

This document examines bacterial leaching with respect to five metals: gold, silver, copper, manganese and cobalt. Current research includes examining the process by which bacteria act to liberate metal from the ores as well as the actual application of this technology on mine sites. However, before a technology is considered viable, its economic feasibility in relation to conventional technologies has to be examined. Hence we will look at the economics of bacterial leaching and present the results of a previous study carried out for copper and gold as well.

The paper intends to show the advantages of bacterial leaching over other technologies presently used by the mining industries, not only economically, but also in terms of environmental protection. Moreover, developing countries have an especially large role to play in this respect not only because bacterial leaching reduces the need for expensive capital investments but also because it enables extraction from dumps which already exist at mine sites and were previously considered uneconomic to process using conventional technologies.

## 2. Bacterial leaching

Bacterial leaching, although we are only now becoming familiar with it, has occurred in nature for hundreds of years. In fact the earliest recorded case of bacterial leaching at a mine site was in Rio Tinto in Spain some 300 years ago. 1/ The bacteria which thrive in the somewhat acidic waters of mines function as oxidizing agents and obtain energy for growth through the oxidation of iron and sulphur. These microbes require the fulfilment of certain conditions to ensure their survival which in turn facilitates bacterial leaching. These conditions include ample amounts of oxygen, a highly acidic pH and specific nutrients. 2/ While the process is ancient, scientists have begun examining the process only very recently. In fact research on applications of bacterial leaching to copper and uranium recovery led to a Kennecott Copper Corporation patent only as late as 1958. 3/

Today, this process is being studied more closely by the scientific community and interest in mining companies has also increased due to lower prices during the late 1970's and early 1980's. The discovery of bacteria which are known to oxidise ores other than sulphides has led to research on the recovery of metals other than copper and uranium. Leaching techniques have also become more advanced due to the close relationship between the degree of optimisation and recovery levels. We can now identify three specific methods of bacterial leaching at mine sites:

### Dump leaching

At present, much of the ore which is retrieved to the surface from mines and is considered to be waste material is left lying in dumps. Sulphide ores can be leached from these waste dumps by spraying a slightly acidic solution containing bacteria. The solution percolates through the dump, dissolving

sulphides in the process, to produce an effluent. The metal can be recovered from the effluent when the mineral concentrations of the solution are high enough (around 2g/litre), by precipitation on scrap iron or through more efficient solvent extraction and electrowinning techniques.

The rate of metal recovery is probably the lowest for this kind of leaching (usually less than 40 per cent of total metal content recovered), because of the lack of optimization. The dumps used for bacterial leaching are not specially constructed for this method of mineral recovery. For efficient recovery, not only do the bacteria have to be kept in optimized conditions, but so does the ore. In the case of dump leaching, the solution is unable to reach the centre of the dump and therefore unable to efficiently leach the entire ore heap. However, as a result of this, the costs associated with dump leaching are generally low with additional costs imposed by the introduction of new equipment such as acid resistant pipes, pumps and collecting tanks. The total costs associated with dump leaching usually range from between US$1 million and US$2.5 million depending upon factors such as dump dimensions and topography. Operating costs are minimal, an efficient process resulting in self generation of acid and no additional purchase of energy. 4/

Heap leaching

As its name suggests, this process involves the leaching of marginal ores during on-going operations and of overburden from newly developed open pit mines, in heaps designed to be constructed and operated according to parameters for optimal bacterial activity at the mine sites. Initial capital and operating costs are therefore higher than they are for dump leaching but as a result the recovery of metal is considerably higher, ranging from between 40 and 80 per cent depending upon the extent of optimization.

Initial costs are normally associated with the designing and optimization of heaps which facilitate efficient aeration and temperature control as well as extensive testing to maximize metal recovery. Higher capital costs are also required for blasting and crushing of ore the extent of which depends upon the natural particle size of the ore. Investment costs for heap leaching have been estimated at between US$5 million and US$50 million. 5/

Concentrate leaching

Also known as vat leaching, this method of bacterial leaching is the most capital and skill oriented. Leaching of ores takes place in confined and optimised environments, allowing more control over the process. This method in the future has the potential to provide an alternative to environmentally damaging processes such as smelting and roasting. As yet however, not enough is known about the microbiology and genetic make up of the bacteria involved and the development of this method is still in its early stages with a few companies such as Giant Bay Resources Inc. and Coastech Research Inc. carrying out experiments in confined environments where tight controls can be maintained. 6/

## 3. Biotechnology applications in mining

### 3.1 Gold and silver

Much of the world's current research in bacterial leaching has concentrated on gold. The precious metal has seen a fairly explosive price rise in the last decade or so. The reason we include silver in the analysis together with gold is that the two metals are almost always found together in nature. It is therefore difficult to study bacterial leaching of silver without mentioning gold and vice versa.

Bioleaching of gold especially certain types of mineralisation is now being commercialised by both biotechnology firms as well as by mining companies all over the world. Gold occurs in rocks either in nugget form or as an inclusion into sulphide minerals such as pyrite, arsenopyrite, pyrrhotite, galenite, sphalerite and silicate minerals. Bacterial leaching as well as other forms of processing have proved popular with gold which has seen its price rise almost continuously since the abandonment of the gold standard system in the 1970's. However, it is in the area of refractory gold that bacterial leaching has shown its true potential.

The reasons for refractoriness in gold may be many: inclusions of submicron gold in sulphide minerals, especially pyrite and arsenopyrite; the presence of iron, copper or lead minerals which consume cyanide by forming metal-cyanide complex ions are some. 7/ These refractory ores do not react to conventional cyanidation procedures. Instead, it has been suggested that pretreatment with bacterial solution makes it possible to recover gold through cyanidation or cyanidation-CIL. Successful applications of bacterial leaching to refractory gold have been demonstrated a number of times both through laboratory testing as well as applications. 8/

Most of the work in this area has concentrated on the use of the mesophilic bacteria T. ferrooxidans which obtains energy from the oxidation of ferrous iron and reduced sulphur compounds. Refractory gold bearing sulphide minerals such as arsenopyrite and pyrite can therefore be oxidised by T. ferrooxidans to recover gold. 9/ However, Coastech Research in Canada has begun investigating alternative bacteria which may be better applied to the leaching process. One of these which was isolated in 1972 and is receiving considerable attention is Sulfolobus acidocaldarius.

In a study published in 1986, researchers from the University of Warwick have tested and discussed the advantages of this bacterium. 10/ Sulfolobus-like bacteria were obtained via pyrite enrichment cultures of Icelandic hot spring and English coal pile samples. Chalcopyrite, pentlandite, pyrite and nickel containing pyrrhotite concentrates were degraded during the autotrophic growth of iron and sulphur oxidising strains of the bacterium at temperatures of 70 degrees celsius. Preliminary results were encouraging for sulfolobus showing that while T. ferrooxidans initially produced better oxidation kinetics, oxidation rates for sulfolobus increased with increasing pulp density up to the highest density.

More recent work studying the leaching of arsenopyrite using sulfolobus at high temperatures at the University of Umea reveals that the bacteria produced stable levels of metal dissolution and promising leaching rates at temperatures of as high as 70 degrees celsius. 11/

Another thermophilic bacteria, Sulfolobus brierleyi was also tested, this time on pyrite leaching. 12/ Results showed that the total amount of iron released by T. ferrooxidans

at 37 degrees celsius was considerably lower than the amount of iron released using S. brierleyi at higher temperatures of 60 and 68 degrees celsius. Similarly, Norris and Barr 13 from the University of Warwick confirmed the ability of thermophilic bacteria such as Sulfolobus to leach pyrite at high temperatures. However, bacterial activity is reduced when mineral concentrations are increased. For this reason, further research needs to be carried out on the screening and study of different thermophilic strains with respect to their tolerance of high solid concentrations as well as improvement in the design of reactors.

Because of the importance of gold as a metal as well as the occurrence of silver together with gold, bacterial leaching research has not concentrated much specifically on silver recovery. This was partly due also to the fact that previous tests of microbial leaching of silver proved to be unsuccessful. 14 According to Ehrlich 15 a probable reason for this lack of success of leaching of silver containing sulphide ores is that silver ion is perceived to be very toxic to micro-organisms in general, discouraging their use in bioleaching of silver ores. Ehrlich however obtained significant results in his test studying the leaching of silver from a mixed sulphide, silver containing ore.

Two samples of ore from the same orebody in Idaho were used, one for a batch leaching experiment and the other for continuous leaching. The culture used in the experiments was a strain of Thiobacillus ferrooxidans. In the case of the batch leach tests, pairs of Erlenmeyer flasks were used, one inoculated with a solution containing T. ferrooxidans. Similarly, for the continuous tests, two reactors were used, one inoculated and the other uninoculated. Three different media containing different levels of iron were introduced. The results of the batch leaching show that of the three media, 9K Fe medium was by far the most effective, T. ferrooxidans accelerating the leaching of Ag, Cu and Zn in 9K and 0.9K Fe medium but in 0K medium its effect was slight for Cu and Zn and absent for Ag.

In the case of continuous leaching the overall rates of silver recovery in the reactor containing T. ferrooxidans were satisfactory (77.5 per cent). Moreover, leaching rates in the continuous process appear to have been relatively selective for silver, the final results showing relatively smaller levels of other metals including copper, zinc and lead recovered in the inoculated continuous test reactor as compared to the inoculated batch test results. This selectiveness may have some practical implications: firstly, depending upon the recovery process, it would facilitate the recovery of silver from the pregnant solution by lessening interference from Cu and Zn, and it helps to preserve much of the Cu and Zn in the ore for subsequent extraction by batch leaching or other suitable processes.

Another series of experiments carried out recently shows that the use of T. ferrooxidans to treat refractory gold arsenic concentrates by tank leaching is much more profitable than alternatives such as cyanidation and autoclave leaching. Moreover, bacterial leaching is environmentally safe when compared to conventional processes such as pyrometallurgical processing. 16 The rate of recovery in the controlled laboratory conditions was as high as over 90 per cent for gold and 80 per cent for silver from the concentrates. Processing in a closed system also reduced the danger of environmental pollution from the substances released during leaching.

Commercial applications of this form of recovery have increased considerably in the last five years or so. In Canada bioleaching plants have been run successfully by Giant Bay Resources Inc. The effectiveness of this process has been demonstrated by a number of pilot scale tests. Bench scale tests initially tested approximately thirty different concentrate samples from sites in North America and Australia to determine their response to bio-oxidation. Of these samples, three were used in continuous bench scale analysis. Finally, a mixed pyrite-arsenopyrite concentrate from Eastern Canada was used in a pilot plant operation which lasted from August to December 1985.

The results during the three stages of operation showed cumulative sulphide oxidations of 62 per cent after stage one, 78 per cent after stage two and 94 per cent after stage three. Some of the advantages offered by bacterial leaching: operation at room temperature and pressure; efficient use of oxygen from air as the oxidant; and disposal of iron, arsenic and sulphur as environmentally safe products. 17 Since then, Giant Bay has been involved in a number of commercial applications, most of them joint ventures using biotechnology on a trial basis. Most of these ventures have involved sulphide gold deposits in Australia and Canada. The world's first gold dore bar was recovered from the treatment of refractory gold ore by bioleaching in September 1987 18 at the Salmita gold mine in the Northwest Territories in Canada.

In the case of Equity Silver Mines Ltd's open pit in British Columbia, the company conducted a feasibility analysis for bacterial pretreatment of the ore by constructing both laboratory and pilot scale bioleaching test facilities. 19 Preliminary laboratory testing of the ore was carried out at BC Research's laboratories. Batch testing revealed that gold recovery by cyanidation appeared insensitive to the degree of pyrite oxidation. However, silver as confirmed by earlier studies, appears highly dependent on Fe extraction. Thus, depending upon parameters such as the price of silver, the tests indicated that it may be advantageous to minimise the degree of oxidation at the expense of silver recovery, to minimise treatment costs of byproduct bioleachate constituents.

The pilot plant was set up mainly using the same parameters as for the batch tests. The results show that although a combined Fe + As extraction of 80-90 per cent is not justified for additional gold recovery which appears to remain constant after about 80 per cent extraction, silver recovery continues to improve as sulphide oxidation continues. The final result at Equity was the setting up of a 2 tonne per day pilot scale trial where the bioleach circuit sizing and operating parameters were maintained at 80 90 per cent combined Fe + As oxidation as silver prices were not high enough to warrant the additional capital and operating costs associated with increased silver recovery.

Newmont Gold has recognised the importance of bacterial leaching for recovery of refractory gold such as found in the orebodies of the Carlin Trend. Ongoing research on the use of the microbe Thiobacillus ferrooxidans and other similar bacteria has resulted in the setting up of a series of pilot facilities for bioleaching. 20

Although most of the commercial applications discussed thus far have concentrated on developed countries, bioleaching of refractory gold and silver

has a great potential for developing countries as well. One of these countries where research in bacterial leaching for refractory ores seems to be relatively advanced is Zimbabwe. Zimbabwe has a number of refractory gold and silver deposits and interest in bacterial leaching has led to considerable research in this field. The Institute of Mining Research at the University of Zimbabwe has examined the potential use of biotechnology in two recent reports. 21

In laboratory testing, a dynamic system was designed for gold concentrates. The first experiment was performed at a 5 per cent pulp density on an arsenopyrite concentrate from the Vubachikwe mine containing 5.5 g Au tonne after cyanidation. The recovery of gold after bacterial leaching increased from 4 to 75 per cent. From these results it was proposed that a continuous leaching process be designed and tested to confirm these results.

In a second experiment, refractory gold concentrate consisting mainly of arsenopyrite was tested from the Bat 20 mine, Gwanda. The results of this experiment showed a 67 per cent oxidation of sulphides in fifteen days and an improvement in gold recovery from 30 to 80 per cent. This was followed up by a final experiment at Broomstock Concentrate Pwe'we. Here, the global oxidation of sulphides was around 55 per cent and gold recovery increased from 45 to 78 per cent. The promising results obtained from these experiments have led to the conviction that while further work is needed to enlarge the scale of bacterial leaching, bio-oxidation has an important potential in the treatment of refractory gold ore in the context of Zimbabwe.

Similarly, the epithermal deposits of the Pacific rim contain refractory ore for which bacterial leaching has potential. The recently developed Porgera mine in Papua New Guinea contains ore which is refractory in nature. Depending on the circumstances particular to this mine, bacterial leaching if viewed as an alternative to pressure oxidation as a form of pre treatment before cyanidation, might prove to be cheaper and more effective. The gold project still in its evaluation stages on Lihir Island once again has refractory ore which needs to be pre treated before the gold can be extracted. In feasibility analyses bacterial leaching should indeed be examined along with all the other alternatives. However, this remains to be seen since mining companies tend to be conservative in the adoption of new technologies. In fact, in a recent survey of copper and gold deposits in Papua New Guinea, bacterial leaching does not even get a mention as a possible alternative to other pre treatment methods for refractory ore. 22

3.2. Copper

Being one of the most important base metals, copper is especially important to developing countries since some of the largest producers of copper in the world are developing countries. In addition copper is especially interesting in relation to this study because of the enormous progress made in bacterial leaching especially by developing countries. Copper is contained mainly in sulphide ores. Unlike gold, most of the bacterial leaching research for sulphides concentrates on dump and heap leaching which required less sophisticated technology and have lower capital and operating costs than concentrate leaching which is used for gold. Because of this, laboratory testing as well as commercial applications of sulphide leaching are becoming more and more common in developing countries as well.

More recently research has developed to the stage of examining continuous leaching systems which would be more desirable for industrial applications. One such study of bacterial leaching of copper sulphide ores 23 shows that T. ferrooxidans provides high copper extraction rates in all batches tested except for those containing Chalcopyrite. This may be due to the formation of iron precipitates on the surface of the chalcopyrite particles, inhibiting the growth of bacteria. Nevertheless, the high recovery rates of 65 to 84 per cent show that bacterial leaching is a viable alternative to conventional technology.

North America, especially Canada, has been at the forefront of research on bacterial leaching of sulphide ores. Canadian universities supported by the mining industry have carried out a considerable amount of research on the various advantages and difficulties posed by different bacteria in biotechnology. The Flin Flon mine in Manitoba has been the subject of two separate studies. 24 Both studies used sulphide ores from the Flin Flon mine to carry out shake flask experiments using T. ferrooxidans and T. thiooxidans in different cultures. Both have concluded that the highest rates of metal recovery are seen when T. ferrooxidans and T. thiooxidans are combined in the leach solution.

A recent project at the Flin Flon mine was initiated by the Hudson Bay Mining and Smelting Company. 25 The results again show the most efficient recovery of metal using mixed cultures of T. ferrooxidans and T. thiooxidans. Hudson Bay Mining and Smelting is currently using the data obtained from shake flask and column tests to determine the cost of leaching the experimental block of mined-out and backfilled stopes at the mine. If these experiments appeared to be economically feasible, the company was expecting to set up a pilot leaching plant.

Experiments with biotechnology have recently been carried out in South west Spain. 26 The ores of the region, which contain substantial amounts of copper, lead, zinc, silver and gold are currently crushed and concentrated by differential flotation. Because of the pyrite content, another alternative is to produce bulk concentrates which can then be treated by a hydro or pyrometallurgical process. Bacterial leaching is considered to be a cheaper and more efficient alternative to these processes. The experiments using T. ferrooxidans show that leaching in the presence of bacteria was faster and more effective than in their absence. Similar leaching experiments using T. ferrooxidans and T. thiooxidans have been carried out in Finland. 27

The relatively low prices of copper in the last decade have prompted more research into in place or in-situ bacterial leaching. It has also been encouraged by the US Bureau of Mines as well as the Canadian Department of Energy, Mines and Resources (CANMET), the latter contracting the mining company Noranda to carry out a pre feasibility study of in situ leaching of copper. 28. One of the mines owned by Noranda is already involved in bacterial leaching. Lakeshore mine in Arizona, USA which in 1983 changed from block cave mining vat leaching of its oxide deposit to a bore hole in place leaching of low grade ore, is currently recovering approximately 700 tonnes per month of copper from solutions assaying about 1.7 g L copper.

The mine chosen by Noranda for study was the Gecco Mine in Ontario, Canada. Unfortunately the results were not encouraging for bacterial leaching

whose cost varies from mine to mine because of different mining environments.

Recently, an in-situ pilot plant was set up at the San Valentino di Predoi mine in Northern Italy. 29/ Initially, shake flask tests were carried out using T. ferrooxidans strains isolated from mine waters. The results confirmed a high leaching rate and accordingly, a suitable bioleaching flowsheet was devised which has since been implemented.

In South Africa, large-scale bacterial tests were carried out in-situ at the Prieska Copper-Zinc mine. 30/ In laboratory tests, leaching of zinc was far better than copper from Chalcopyrite. Despite the poor results obtained for copper, zinc recovery was high enough to encourage continued large-scale laboratory tests. However, here too, as in Canada, it was decided that in-situ bacterial leaching would not be a feasible alternative because of problems of adequate access for distribution of the lixiviant and the dimensions and attitude of the orebody.

In the developing world too, work on bacterial leaching of sulphides is continuing. The Andean Pact countries are possibly the leaders in the use of this technology in the Third World. As has been noted before, 31/ several bacterial leaching industrial scale operations already exist in developing countries: a dump leaching operation at Bougainville in Papua New Guinea, a combined dump and underground leaching operation at Cerro de Pasco in Peru and a semi-optimised heap leaching operation at Cananea in Mexico. In addition, Centromin of Peru has designed a semi-industrial scale bacterial heap leaching plant to extract copper from low grade ore from overburden at Toromocho.

More recently, Mineroperu has submitted a proposal for bacterial leaching of copper for approval. The proposed process to be developed at Mineroperu's Cerro Verde unit will entail treating secondary sulphides by acid-ferric bacterial leaching to produce 15,000 tonnes per year of copper cathodes using existing facilities and floating the fines to produce 57,000 tonnes per year of copper cathodes. 32/

In Chile, laboratory as well as semi-industrial scale tests were conducted to help determine some of the parameters of an optimal bacterial leaching industrial project. 33/ A mixed kinetic model describing the dissolution of low grade copper ores from the El Teniente mine in Chile was recently set up. Predictions from the model when compared with experimental data from a bacterial leaching operation in a pilot column showed surprisingly similar results. 34/ Codelco in Chile has been using bacteria for dump leaching projects: one for treating low grade sulphides (0.2 to 0.5 per cent) and the other for treating coarse middlings from its concentrator. 35/

In Panama, percolation leach testing has been carried out on ores from the Cerro Colorado copper deposit. 36/ The recoveries were poor, jarosite deposition favoured by poor liquor distribution over and in the fairly alkaline rocks was probably a major cause of the cessation of leaching. In contrast, a major success was reported with bacterial leaching of Chalcopyrite concentrates from Mosaboni in India. 37/ The tests were carried out first in shake flasks and then in a glass bioreactor using T. ferrooxidans MCM B 231. The experiments were largely successful, the bioreactor recovery rate reaching 88.64 per cent, significantly higher than recovery rates of 70.24 per cent in shake flasks. This significant improvement appears to be due to efficient aeration and consequent high rates of oxygen and carbon dioxide mass transfer achieved.

### 3.3. Manganese

So far, research on bacterial leaching has only concentrated on the group thiobacillus. Thiobacilli are effective only when applied to sulphide ores. 38/ Much of the world's mineral wealth however, is contained in other accumulations. Manganese for example, can be found in the form of oxides, carbonates and silicates. There are however limits to the use of chemolithotrophic thiobacilli for these ores. This is because the energy supplying substrates (sulphides, iron (II), sulphate and sulphur) are missing and must be added to the ore. Moreover, the pH values (usually greater than 5) in the leaching solution have an inhibiting effect on the bacteria.

Several methods for leaching of manganese ores have been identified thus far. A feasibility test was carried out by the US Bureau of Mines for leaching of manganese from low grade oxide and carbonate ores. The presence of organic material including leaves and yeast resulted in an average leach rate of about 97 per cent after 60 days. 39 This led them to conclude that micro-organisms can be used to leach manganese from oxides and carbonates.

Another method of manganese leaching replaces the sulphur content, which in turn enables leaching by sulphide oxidising bacteria. The method which was patented in Japan, leaches manganese from an aqueous solution of manganese sulphate using bacteria to oxidise sulphur to sulphuric acid. Initially, the bacterium T. ferrooxidans was used to produce sulphuric acid. This proved unhelpful since manganese dioxide is almost insoluble in sulphuric acid. However, in the presence of T. thiooxidans, almost all of the manganese dioxide was converted into manganese sulphate at an extremely rapid rate. 40/

The use of heterotrophic bacteria also aids in the process of manganese leaching. In India, an ore containing 44 per cent manganese was leached using cultures of Pseudomonas sp. and Basillus sp. Precipitation of the solubilized manganese was carried out with the addition of lime resulting in a 90 per cent recovery rate after 90 days. 41/ In the USSR, manganese was recovered using heterotrophic bacteria on liquid wastes. Once again the recovery rate was high, 90-96 per cent in only 12 days. 42/ An important conclusion here is that contrary to conventional beliefs bacterial leaching under certain circumstances may result in high rates of metal recovery in a relatively short period of time.

### 3.4. Cobalt

Cobalt is used mainly for the production of superalloys for use by the aerospace and other industries. In 1988, the two largest producers of cobalt, Zaire and Zambia together accounted for over 70 per cent of total market economy production. 43

Cobalt very often occurs in nature along with other sulphides, predominantly copper and nickel sulphides. Until now more emphasis appears to be placed on the biological recovery of these other metals. The fact that most of the world's cobalt is produced in these two African nations may partly account for the slow growth of bacterial leaching of cobalt. Indeed as we saw, the main reason why gold

has received so much attention is linked to its price as well as the fact that a large amount of gold is produced in developed countries where more advanced technology and environmental legislation has provided incentive for mining companies to branch out into other methods of processing which are potentially cheaper and less harmful to the environment.

Some have also argued that because cobalt is largely produced in areas of the world which are potentially unstable, it is increasingly being regarded as a strategic mineral. 44/ Interest in alternative means of producing cobalt is therefore likely to grow in the near future.

Some progress is already being made in this field. In South Africa, nickel and cobalt can usually be found together. However, although nickel deposits do exist in the country, they are usually low grade and disseminated in nature and with the present economies and technology, their exploitation is considered to be uneconomical. 45/ Nickel in South Africa is principally found in pentlandite which contains quantities of cobalt. Conventional extraction techniques have so far been unsuccessful in extracting a high percentage of metal from this kind of ore in which mineralisation occurs as exsolved fine lamellae in pyrrhotite. The result is a dilemma as to whether grade should be sacrificed in favour of recovery. The South African Council for Mineral Technology (MINTEK) has therefore initiated a study examining other non conventional means for extracting nickel from such ores. The study examined the agitated bacterial leaching of nickel sulphide, producing cobalt and copper as by products.

The semicontinuous process, showed a large increase in extraction rates for both cobalt and nickel after about five weeks, the leaching rate steadying to over 85 and 90 per cent respectively after about 11 weeks of leaching. The authors of this study also designed a flowsheet which would justify bacterial leaching of nickel and cobalt on a practical scale. Although the economics of bacterial leaching for this particular case were not discussed, the authors argued that opinion in the mining industry is continually being adjusted to favour bacterial leaching as we see economic leaching rates which are competitive with more expensive and environmentally detrimental conventional processes.

## 4. Biosorption: The environmental benefits

A relatively new area of research in the field of bioleaching is biosorption, the process by which microbial biomass, living or dead aids in the removal of metals or toxic substances from waste material and industrial effluents. Research in this area ranges from the recovery of precious metals such as gold, 46/ the recovery of cobalt, 47/ copper binding, 48/ to the accumulation of heavy metals and radionuclides by non growing fungal biomass. 49/

At the Homestake gold mine in South Dakota, a process has been developed for the biodegradation of cyanide waste which removes free cyanide and metal complexes from effluent before discharge to the local watercourse. 50/ The biomass that is recovered from bioreactors shows the presence of about 45 grammes per ton of gold. The fact that the presence of gold was not detectable in the feed to biodegradation indicates the extent of the scavenging power of the micro organisms.

The adsorption properties of a bacterium (Bacillus subtilis), a fungus (Aspergillas aiger) and two species of algae (Chlorella vulgaris and Spirulina platensis) were compared. All three are known to adsorb heavy metals. Results showed that the adsorption of gold tended to a maximum in the range pH 3-4 for all four organisms, with Chlorella showing the best results for pure gold chloride (90 per cent adsorption in four minutes at 2 per cent loading). For a potential industrial process, a semi-continuous process of selective gold recovery from dilute solution was also demonstrated using a column of alginate gel immobilized algae which although restricting the adsorption rate, enabled a high recovery of gold with good selectivity.

A similar comparative study looked at the role played by two bacterial strains and a unicellular alga in the adsorption of uranium, silver and gold, from barren solutions, small-scale in situ leachates and waste streams from metal finishing operations. 51/

Apart from their adsorption abilities, these micro-organisms are also important in environmental conservation. Recent EEC directives against the discharge of industrial effluents including air pollutants such as sulphur dioxide produced by smelters as well as environmental legislation in the US and Canada has led to the increasing use of bacteria such as T. ferrooxidans in the mining industry. More recently the importance of harmless algae and fungi which can be manipulated into effective forms for metal recovery from mining operations and industrial effluent has been acknowledged. 52/

One such application is the AMT-BIOCLAIM process which applies biosorption for metals removal from wastewater. 53/ The process which uses a granulated non-living biomass product for a metal removal agent (MRA) was tested on wastewater from a jewellery manufacturer and a manufacturer of precious metal compounds. Both kinds of gold cyanide wastewaters contained either very low levels of free cyanide (as in the case of the jewellery manufacturer's wastewater) or none at all (metal company's wastewater). Despite varying the volume of the sample and the pH level, the level of metal recovery especially that of gold was extremely good for both kinds of wastewaters.

Following up on this, a pilot plant for the removal of lead from an industrial effluent was set up by the owners of this process, Advanced Mineral Technologies Inc. and was run for 39 days. A stable and efficient removal of lead (98-99 per cent) was achieved. The variation in pH did not influence lead removal, nor did the variation of lead content in the effluent which ranged from 0.01 to 4.30 mg/l. 54/

Thus the research which has been carried out in this new area of bacterial activity in the field of metallurgy shows that biosorption has both financial and environmental potential. However, it must be made clear that the field is extremely new, and the processes involved complex. Nevertheless biosorption has a substantial contribution to make both in terms of increasing annual metal production levels as well as providing incentives to companies which are at present required to treat wastewaters as part of the general effort especially in developed countries to clean up the environment.

## 5. Economics of bacterial leaching

Thus far we have shown that the use of bacterial leaching can be beneficial in terms of high recovery rates as well as the important side

effect of reduced environmental pollution. However, before bacterial leaching can be convincingly portrayed as a feasible alternative to conventional processing technologies we have to examine the economic costs and benefits of the technology especially in comparison with some of the older techniques. This section is divided into two parts. The first will present a brief survey on the latest developments in the economics of bacterial leaching. Then we will present the results of an economic feasibility study 55/ which considered orebodies of different sizes and ore grades as well as different forms of bacterial leaching in relation to copper and gold deposits.

5.1. Is bacterial leaching economically viable?

The work in this field initially began with refractory gold and silver since metal is rather difficult to extract economically from this kind of mineralisation using conventional cyanidation processes. Bacterial leaching seemed to be the only alternative and if it could be proved economical, would provide a feasible alternative for extraction of gold from refractory ores.

Preliminary experiments at Giant Bay Inc. with bacterial leaching for refractory gold-silver concentrates 56/ led to an engineering feasibility study by Wright Engineers Ltd. The site chosen was in Northern Ontario and the results from this study would apply only to that site. Capital and operating cost estimates for the BIOTANKLEACH process were prepared at operating rates of 50, 100 and 200 tonnes per day. These were compared to capital and operating costs estimated for roasting and pressure oxidation at a rate of 100 tonnes per day. The results showed that the overall cost of the Giant Bay technique was significantly less than the two alternative technologies.

Similarly, researchers from Davy Mckee and University College Cardiff 57/ compared bacterial leaching of gold from two pilot plants each with a different average grade and capacity with a pilot plant to treat a flotation concentrate without bacterial leaching. The results showed a definite advantage for bacterial leaching prior to cyanidation. However, the results also indicated that bacterial leaching could be most economically viable when the average grade of gold in the concentrate was relatively high (in this case the plant which appeared most economic contained 1.2 g Au/tonne assay). This is similar to the conclusion reached by our study on the economic viability of bioleaching of refractory gold. 58/

A number of others have recently also examined the feasibility of bacterial leaching in comparison with other technologies, both for gold 59/ as well as for copper. 60/ The copper study by BC Research first demonstrated a system of bioleaching of chalcopyrite. While the technical feasibility of bacterial leaching was established by the study, the economic analysis showed that bioleaching was only marginally competitive with conventional processes. However the authors argued that this may change if environmental restrictions forced higher smelting charges. Since the study was completed, environmental restrictions have become considerably more stringent and bacterial leaching may now be considered more competitive.

5.2. Bacterial leaching of copper and gold ores

Before concluding this study, we would like to present the results of a preliminary computer modelling exercise in which we attempted to determine the range of orebody sizes as well as methods by which bacterial leaching would be a feasible alternative to conventional technology. 61/ As an example we used two kinds of orebodies, porphyry copper and refractory gold deposits. However, the model can be changed so as to carry out a similar analysis for other kinds of deposits, including the other metals presented in this study.

5.2.1. Description of Models and Methodology

The cost and revenue models were developed at the Royal School of Mines in London. They were constructed within the MECON system and incorporate models for capital and operating costs, revenue and tax regime. The currency used throughout the study was US dollars. The mineral exploitation models incorporated a data base of cost models which could be selected for the flowsheet cost centres which in turn were based on the unit operations. The various parameters of the flowsheet model can be simply modified to tailor the system to the flowsheet.

Cost Models: The cost models are based on a number of sources. 62/ In these models the capital and operating costs are expressed mathematically in terms of capacity, that is,

$$\text{COST} = A \times \text{CAPACITY}^{B}$$

where A and B are constants. A is the magnitude factor and B is the scaling factor.

The US Bureau of Mines claims a reliability for their cost models of plus or minus 25 per cent. The MECON system has been able to achieve plus or minus 30 per cent. These valuations are of course highly dependent on technical consistency and accuracy where accuracy is dependent on the input.

Revenue Models: Revenue was calculated by applying metal prices to the recovered metal. These required the estimation of in situ mined grade, dilution, recovery, production rate and metal price. We used a geostatistical model to produce the relationship of average grade above the cut off grade to the proportion of reserves above the cut off grade. The model used a log normal distribution with a constant coefficient of variation. In this study, the values used for the coefficient of variation were 0.22 for copper and 0.52 for gold. For simplicity and because of time constraints, we assumed that the copper ore bodies contained only copper and the gold ore bodies only gold.

Taxation: A "typical" tax regime was chosen: a 40 per cent tax rate, a 5 per cent royalty rate and straight line depreciation over 10 years for capital allowance costs. Mining policies such as tax systems and royalty rates play an important role in the economic feasibility of a mining project.

Methodology: The value of the ore body under consideration was measured in terms of the Net Present Value discounted at 10 per cent. Note that the NPV (10 per cent) increases as cutoff grade increases to a maximum, and then falls. The value of an orebody, that is, NPV (10 per cent) for a given capacity was taken to be this maximum value.

A Note On Cut off Grades: The cut off grade is the point at which a distinction can be made between the ore that is processed, and the ore which is considered waste. So for example, if the cut off grade for a particular mine was 0.3 per cent copper, the ore which had an average grade higher than 0.3 per cent copper would be sent for processing and the rest of the ore which had an average grade lower

than 0.3 per cent copper, would then be dumped by the side of the mine as "waste." This relationship between processed ore and waste forms the basis of processing decisions and mining companies spend a considerable amount of time and capital ascertaining the "optimal" cut-off grade which varies from mine to mine.

Changing the value of the cut-off grade will bring about a number of changes in processing and the annual value of the final product. In particular, the trade-offs associated with increasing or decreasing the cut-off grade are extremely important in a mining operation. In this study for e ample, we found that for copper, because a typical coefficient of variation for the grade is low (0.22), the range of influence of the cut-off grade is relatively small compared to the case of gold where increasing or decreasing the cut-off grade has a considerable impact on the size of mineable reserves.

The following were the examples of orebodies, average grades and annual production capacity used:

Copper

(i) Dump leaching for reserves of 50 million tonnes, at an average grade of 1 per cent copper with an annual capacity of 3 to 8 million tonnes.

(ii) Dump leaching for reserves of 250 million tonnes, at an average grade of 0.5 per cent copper with an annual capacity of 12 to 22 million tonnes.

(iii) Heap leaching for reserves of 50 million tonnes, at an average grade of 1 per cent copper with an annual capacity of 3 to 8 million tonnes.

(iv) Heap leaching for reserves of 75 million tonnes at an average grade of 1 per cent copper with an annual capacity of 5 to 9 million tonnes.

(v) Heap leaching for reserves of 150 million tonnes at an average grade of 1 per cent copper with an annual capacity of 6 to 12 million tonnes.

(vi) Vat leaching for reserves of 150 million tonnes at an average grade of 1 per cent copper with an annual capacity of 6 to 12 million tonnes.

Gold

(i) Vat leaching for reserves of 10 million tonnes at an average grade of 3 gm Au/tonne with an annual capacity of 0.5 to 0.9 million tonnes.

(ii) Vat leaching for reserves of 10 million tonnes at an average grade of 4 gm Au/tonne with an annual capacity of 0.5 to 0.7 million tonnes.

(iii) Vat leaching for reserves of 10 million tonnes at an average grade of 5 gm Au/tonne with an annual capacity of 0.5 to 1.0 million tonnes.

(iv) Heap leaching for reserves of 10 million tonnes at an average grade of 3 gm Au/tonne with an annual capacity of 0.5 to 0.9 million tonnes per annum.

5.2.2 Results

Copper

The results of the two dump leaching tests showed that while (i) was economic, having a positive NPV (10 per cent) for all capacities, (ii) was economic only for capacities larger than 14.5 million tonnes per year. For the heap leaching scenarios (iii), (iv) and (v), it was found that they were economic only at ore capacity levels of over 50 million tonnes.

The final computer analysis for copper looked at bacterial vat leaching at 1 per cent copper grading of an orebody of 150 million tonnes. However, while the same scenario was successful using bacterial heap leaching (see above), bacterial vat leaching was not economic for copper. Values for the first dump leaching scenario showed the orebody to have a positive NPV for all values. However in the case of the second dump leaching analysis NPV was positive only for values above 14 million tonnes per year. In the case of heap leaching, while the two larger sized orebodies of 75 million and 150 million tonnes were economic at all capacities, the smallest reserve of 50 million tonnes was not economic at all, that is, it had a negative NPV for all capacities. Indeed vat leaching of copper was not economic at all and because of the large negative values obtained, this method of leaching was rejected altogether for copper orebodies.

Gold

Three different scenarios using bacterial vat leaching for different average ore grades were considered. While (i) was uneconomic, (ii) was only marginally economic. However (iii), which considered high grade refractory gold ores of 5 g Au/tonne, proved to be economic. In the case of gold we saw that as the cut-off grade rose the NPV of the orebody rose until a maximum value was reached and then began to slope down. This maximum point is the optimal cut-off point and demonstrates the maximum value of NPV for the project. This value was positive for the highest grade of gold chosen, 5 g Au/tonne, only marginally economic for 4 g Au/tonne and negative at all points for the lower grade, 3 g Au/tonne.

This suggests that heap leaching can be more effective for lower average grades of refractory gold than vat leaching. In contrast, the single analysis using heap leaching showed that it was marginally economic using lower average grades of 3 g Au/tonne.

The results of this analysis, although preliminary, showed that bacterial leaching can indeed be considered a viable technology for certain orebodies depending upon size of reserves, average grades of mineralisation as well as cut off grades. It must however be stressed that in the final analysis, the costs and benefits of bacterial leaching will differ from mine to mine and while in some cases will be considered economic compared to other technologies, for others it may not. What this study does do is provide an example in which bacterial leaching did provide economic benefits for a range of orebodies with differing average grades. The initiative of introducing this new technology as a possible alternative in feasibility analyses must now be taken by the mining community which in some cases is already taking place.

## 6. Conclusions and suggestions for future research

This study was based on a review of the latest developments in the field of bacterial leaching especially in relation to five metals: gold, silver, copper, manganese and cobalt. While copper and the precious metals, gold and silver have received the most attention so far, applications to other metals such as the two identified above are also progressing.

We reached three major conclusions from this study and these may also provide research areas for the future:

1. With pressure on countries, especially developing countries, to adjust to changing conditions in the mining industry and international markets, bacterial leaching offers considerable potential. Bacterial leaching offers developing countries the ability to develop their own capabilities in the area of biotechnology. Having developed this capability in bacterial leaching, developing countries can compete with developed countries and among themselves to expand economically, not only in mining but also in other areas where biotechnology has been applied with considerable success.

2. Bacterial leaching is an environmentally sound technology. Mineral extraction processes currently make use of smelters which are not only expensive to construct but are also a major source of environmental pollution. The world has recognised the importance of environmental conservation as is evident from recent developments in industrial countries. Bacterial leaching not only provides an alternative, environmental friendly technology to mining companies in the north who are bound by environmental regulations but also provides developing countries with a new technology which is cheaper than and at least as efficient as smelters while at the same time bypassing some of the environmental damage caused in the north. The new area of biosorption also promotes the use of biotechnology in solid waste treatment substantially reducing pollution from mining and industrial effluents.

3. The results from the economic feasibility analysis for copper and gold bioleaching show that for copper, while dump leaching was economic for lower sized orebodies, heap leaching was successful for orebodies of a larger size. This is an important conclusion, since metal recovery using heap leaching can be significantly higher by optimising leaching conditions than in the case of dump leaching where recovery levels cannot be predicted but are generally lower. Additionally, this is important for developing countries such as Chile, one of the main copper producers, where the size of deposits generally tends to be large. (Porphyry copper orebodies in the country range from about 4.6 million metric tonnes at the El Indio copper mine to 18.5 billion metric tonnes at Chuquicamata, the world's largest copper deposit.)

For gold, we can conclude that this preliminary analysis favours the use of heap leaching for lower grades while vat leaching can be used for higher grade ores. Vat leaching which requires optimization in controlled environments is generally considered to be unfavourable for developing countries which may not have access to the optimisation facilities or the higher capital investment associated with this form of bacterial leaching. Heap leaching on the other hand, as already pointed out, can result in metal recoveries of up to 80 per cent when optimised.

### 6.1. Advantages of Bacterial Leaching for Developing Countries

It is perhaps to developing country metal producers that biotechnology provides the greatest advantages. The technology requires relatively lower capital investment and operating costs are also low when compared to conventional mining. Biotechnology may also prove useful in solving a number of metallurgical problems associated with complex ore deposits, many of them found in developing countries. In this respect it has already demonstrated its potential to extract different metals such as the separation of zinc and lead concentrates from these multimineral deposits.

Research conducted in developing countries has demonstrated the applicability of bacteria to the leaching of copper and refractory gold. Still further potential lies in the refractory deposits of the Pacific rim countries especially Papua New Guinea. For the other metals, research is still in its preliminary stages, however, since a large percentage of both but especially cobalt is produced in developing countries, applications of bacterial leaching to these metals will be especially beneficial to developing countries.

As much of the current bacterial leaching applications are carried out on ore dumps, developing countries which have higher grade ore dumps (less sophisticated technology in developing countries has imposed a higher cut-off grade in mining operations. As a result, ore dumps have a higher metal content in developing countries than similar dumps in developed countries) are in an especially good position to use bacterial leaching technology.

In developing countries the cost of building refining plants is enormous. In building a smelter, the relevant mining authority has to take into consideration all the extra costs such as the cost of transporting ore from mines all over the country to be processed at the smelter. As a result, poorer developing countries tend to export unprocessed minerals to countries which do have the facilities for refining ore (mostly developed countries). One such example is Zaire which ships its raw materials to Belgium to be refined. In this process, the developing country loses the value added from refining the metal. Bacterial leaching, by enabling the production of a purer form of metal at the mine site, again offers an advantage in this respect. Thus optimisation of the leaching process provides developing countries with the opportunity to lower costs, mine a larger range of metals and increase their value added by producing a marketable final product.

Thus biotechnology, although used extensively in industry and agriculture is relatively new to mining. Considerable research still remains to be done to exploit this technology effectively. Despite

this, the few commercial applications that have developed over the last few years have demonstrated its effectiveness. Increasing interest on the part of the international conglomerates and governments promises further developments both in research and industrial scale applications of bacterial leaching to the mining industry.

**NOTES**

1/ LeRoux, N. W. (1969).

2/ Warhurst, A. C. (1985b), pp 6-7.

3/ Harris, L. and J. A. Brierley (1989), pp 301-304.

4/ Warhurst, A. C. (1989), pp 6-7.

5/ Ibid., pp 8-10.

6/ See for example McElroy, R. O. and A. Bruynesteyn (1978) and Hackl, R. P., F. Wright and A. Bruynesteyn (1986).

7/ Monhemius, A. J. (1987), p 1.

8/ A number of studies have demonstrated the success in using bioleaching for pre-treatment of refractory gold. These studies indicate that bacterial leaching has definite advantages over conventional pre-treatment methods. See for example: Hackl, Wright and Bruynesteyn (1986), Hackl, Wright and Bruynesteyn (1987), Lawrence (1987) and Gilbert, Bounds and Ice (1987).

9/ Lawrence, R. W. and P. B. Marchant (1988).

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29/ Rossi, Trois and Visca (1986).

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31/ Warhurst (1985b), pp 100-103.

32/ Anon (1988), Mining Annual Review, p 304.

33/ Pincheira, et al. (1986).

34/ Castillo, Herrera, Neuberry, Vargas, Wiertz and Badilla-Ohlbaum (1988).

35/ Ismay, Rosato and McKinnon (1986).

36/ Choi and Hopkin (1988).

37/ Khinvasura and Agate (1988).

38/ Bosecker (1987), p 367.

39/ Karavaiko et al. (1988), p 306.

40/ Murr, Torma and Brierley (eds) (1978), p 278.

41/ Karavaiko et al. (1988), p 307.

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46/ Brierley, Brierley and Goyak (1986), Kuyucak and Volesky (1987) and Kuyucak and Volesky (1988).

47/ Kuyucak and Volesky (1988).

48/ Treen-Seers, Martin and Volesky (1986).

49/ Gadd, While and de Rome (1988).

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| 016 | Machinery | 028 | Promotion of export oriented industries | | |
| | | 029 | Industrial development surveys | | |

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