OCCASION

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

DISCLAIMER

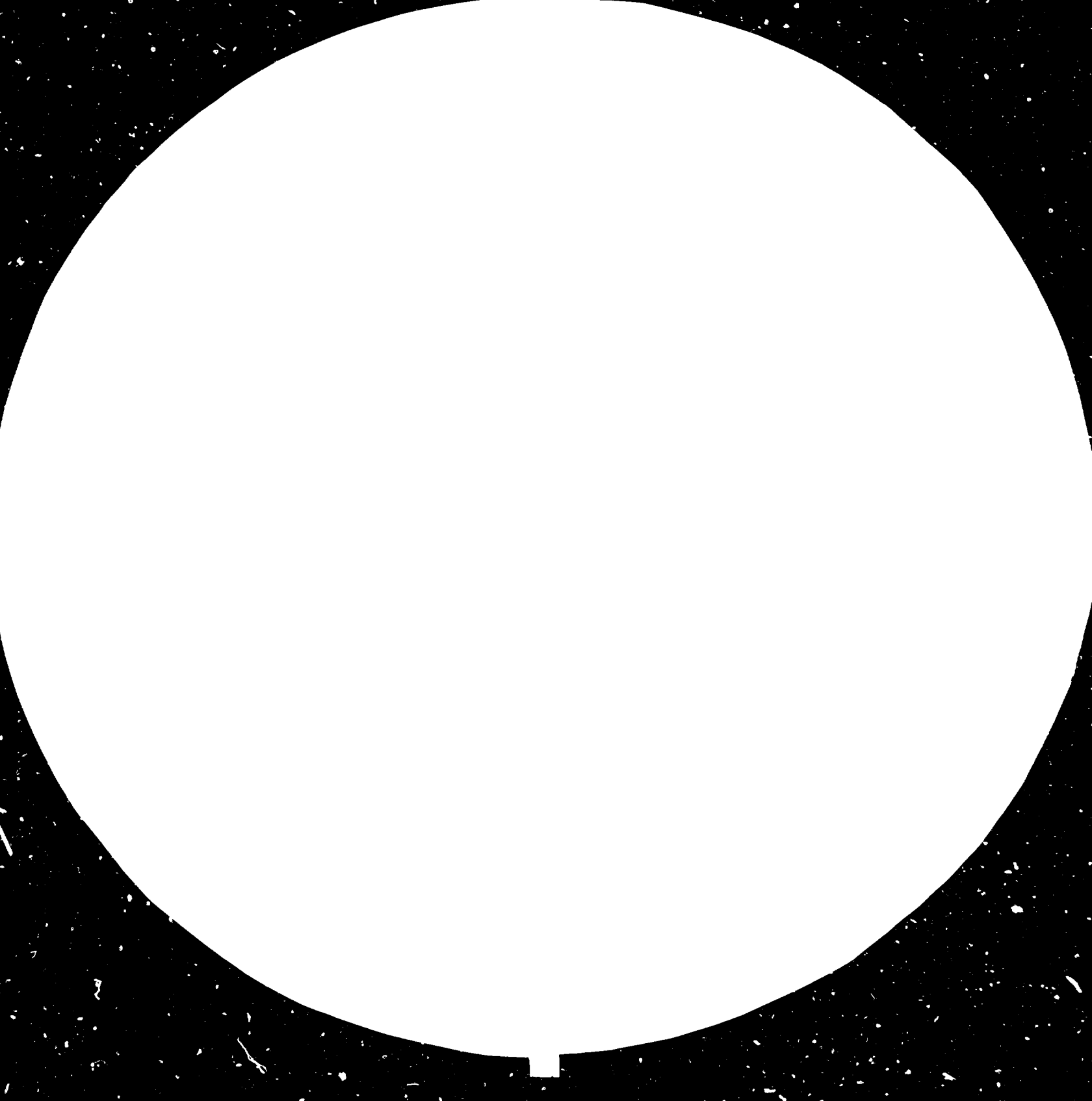
FAIR USE POLICY

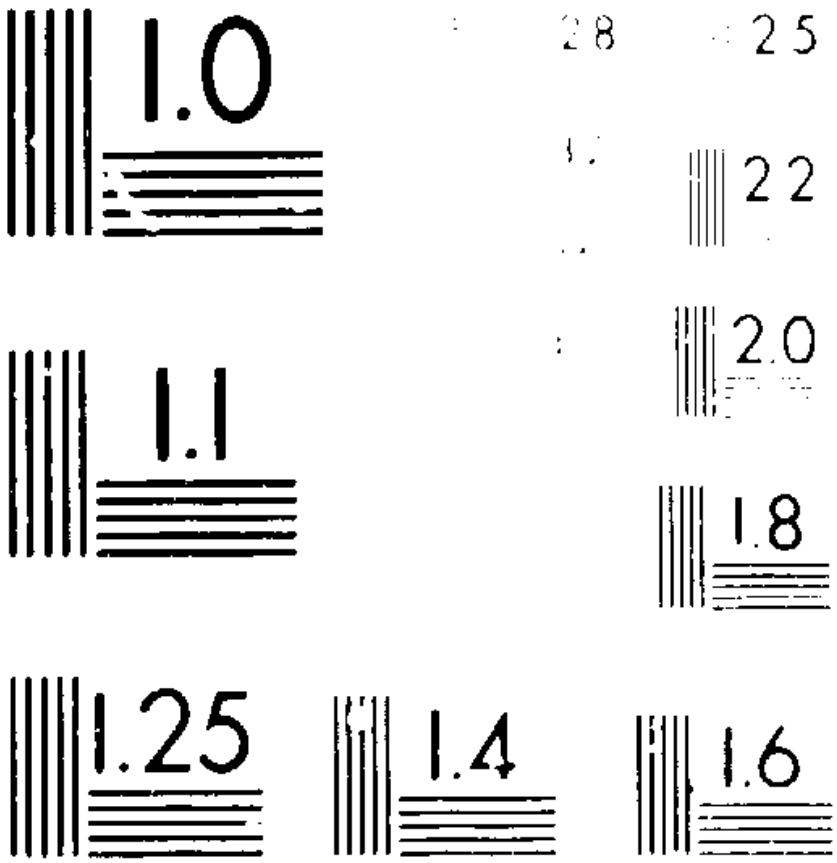
CONTACT

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

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

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




2.8
2.5
1.0
2.2
2.0
1.1
1.8
1.25
1.4
1.6

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

Genetic Engineering and Biotechnology Monitor

Issue Number 7

13218

November 1983

Dear Reader,

The agreement reached in Madrid on the Statutes of the International Centre for Genetic Engineering and Biotechnology came as a matter of great satisfaction to the distinguished scientists involved in the effort, as well as the UNIDO secretariat itself. Delegates from 50 countries attended the meeting, of which only seven were observers. It was again with some thrill that we witnessed, late on the concluding day of the meeting, plenipotentiaries from 26 countries lining up to sign the Statutes at the meeting itself. This brought home to us quite remarkably that many developing countries, including those which are relatively well advanced in this field, have a great faith in this technology for solving their development problems, and also in the role that international co-operation can play in this respect. The meeting also adopted a recommendation on international co-operation where it agreed that UNIDO should continue its work with other relevant agencies to take steps to help the cause of the Centre.

The Centre, as it emerged from the conclusions reached by the meeting, is an international organization composed of a centre and a network of affiliated national, sub-regional and regional centres. This definition of the Centre in the Statutes verily makes its linkages very broad and thus endows it with a great potential for the future.

One of the scientists who rejoiced at the adoption of the Statutes was Professor Ahmad Bukhari of Cold Spring Harbor. It came as a great shock to us when in November he suddenly passed away. His dedication and commitment was as outstanding as his experience in the field.

After the Madrid meeting the 28 countries who have so far signed the Statutes found themselves to be the Preparatory Committee with the task of tackling the important question on the location of the Centre, a timely decision on which is imperative for its speedy establishment. The Preparatory Committee met once in November and will meet again in January.

In November we also participated in two national level workshops - one in the Republic of Korea and the other in Brazil. You will hear more about these workshops in the next issue.

Now that the Monitor is almost two years old and has been giving a quarterly review of the developments in genetic engineering and biotechnology, it might be useful to make a proper review of what has happened during the last two years in this field. Dr. B. Zimmerman is writing such a review for us and we hope to publish it as a supplement in the next Monitor.

Last, but not least, the Monitor wishes all its readers a happy and prosperous New Year.

G.S. Gouri
Director
Division for Industrial Studies

Compiled by the Technology Programme of UNIDO P.O. Box 300, A-1400 Vienna, Austria

NEWS AND EVENTS

International Centre for Genetic Engineering and Biotechnology

The Ministerial-Level Plenipotentiary Meeting on the Establishment of the International Centre for Genetic Engineering and Biotechnology (ICGEB) was held in two parts at Madrid, Spain, from 7 to 13 September 1983; the first part being a high-level meeting to resolve outstanding issues and the second part being a ministerial-level plenipotentiary meeting to adopt and sign the Statutes establishing the ICGEB.

The first part of the meeting was held from 7 to 12 September 1983. It submitted a report to the second part of the meeting, i.e. the Ministerial-Level Plenipotentiary Meeting, which was held from 12 to 13 September 1983.

Present were delegations of 43 countries, observers from seven countries and observers from interested United Nations agencies and other international organizations.

Statutes establishing an International Centre for Genetic Engineering and Biotechnology were adopted at the close of the meeting by 25 countries, namely Afghanistan, Algeria, Argentina, Bolivia, Bulgaria, Chile, China, Congo, Cuba, Ecuador, Egypt, Greece, India, Indonesia, Italy, Kuwait, Mauritania, Mexico, Nigeria, Spain, Sudan, Thailand, Trinidad and Tobago, Yugoslavia and Zaire.

According to the statutes, the Centre will promote international co-operation in developing and applying peaceful uses of genetic engineering and biotechnology, especially for developing countries. It will assist them in strengthening their scientific and technological capabilities in the field, helping with activities at regional and national levels. In this regard, the Centre is expected to act as a focal point for a network of affiliated research centres. Existing national, subregional, regional and international networks will serve as "affiliated networks". The Centre will also serve as a forum for exchange of information among scientists of member states.

Aside from R and D, training of scientific and technological personnel from the third world, both at the Centre and elsewhere, will be one of its main functions. Advisory services will be provided to members to develop national technological capabilities. Among the Centre's other functions are a programme of bioinformatics and collection and dissemination of information.

All rights deriving from the Centre's work will be vested in the Centre, the policy being to obtain patents and interests on patents on results of its projects. Access to intellectual property rights will be granted to members, as well as to non-member developing countries in accordance to international conventions.

A Board of Governors, consisting of a representative of each member together with the Head of UNIDO serving in an ex-officio capacity, will set general policies and principles. It will admit new members and approve the work programme and budget, among other tasks. A council of scientific advisers will be set up, consisting of up to 10 scientists and technologists elected by the Board. Its responsibilities include examination of the draft work programme and budget, as well as reviewing implementation of the approved work programme. A director, appointed by the Board, will serve as chief scientific/administrative officer, legal representative of the Centre and secretary of the Council.

Financing for the Centre will mainly come from initial contributions for its establishment, annual contributions by members and voluntary donations. Least developed countries may become members of the Centre on the basis of more favourable criteria to be established by the board. The regular budget of the Centre during the first five years will be based on the amount pledged annually by each member for those years. After this period assessed annual contributions may be considered.

The statutes will enter into force when at least 24 states have deposited instruments of ratification and sufficient financial resources are ensured.

Although consensus could not be reached on selecting a site for the Centre, the meeting agreed on the creation of a committee to further preparatory work for the Centre's eventual establishment. It will be composed of States that have signed the statutes until their entry into force. Among its tasks is examination of the location of the Centre "in different locations and components", to be completed by 31 January 1984. The resulting report will be circulated to all interested governments, with the committee possibly recommending a date and place for reconvening of a plenipotentiary meeting.

The meeting also adopted a resolution on international co-operation for strengthening developing countries' technological capabilities in genetic engineering and biotechnology. All countries were urged to become members of the Centre as soon as possible, while the international scientific and technological community was asked to assist the Centre. UNIDO was requested to continue to aid in establishment of the Centre as well as to help the preparatory committee in its work.

Governments were invited to make voluntary contributions for facilitating UNIDO's work in this field. United Nations bodies were called on to continue their support for the Centre's establishment. They and other regional, international and non-governmental agencies were asked to contribute financially to activities in biotechnology and genetic engineering.

The President of the ministerial-level session was Jose Maria Maravall Herrero (Spain) and of the high-level session was Emilio Muñoz Ruiz (Spain). The Vice-Presidents were J.K. Kimani (Kenya), Rudolfo Gonzalez Guevara (Mexico), Richard Bouveng (Sweden), F.B. Straub (Hungary) and Hu Zhaosen (China). The Rapporteur was Nizar Mulla Hussein (Kuwait).

Global gene bank, convention are urged for plant resources

An effort to achieve an international agreement that would provide for the conservation of the genetic resources needed to develop higher-yielding crop varieties and that would protect the distribution of such breeding material from restrictive practices faced a major test at the FAO Conference last November. Conference delegates had before them a proposal for the establishment of an international gene bank and the "elements" for a draft international convention on plant resources, both of which were requested in a resolution of the previous Conference in 1981.

This document was originally prepared by the FAO Secretariat. Last March FAO's Committee on Agriculture asked the Director-General for further elaboration on some aspects, following the advice of a special working party, before submitting the study to the Council and the Conference in November. The many and divergent views expressed in that first preliminary public discussion of the subject reflect a relatively new interest and concern for plant genetic resources.

Plant breeders received due credit - and it seemed that properly funded plant genetic research could be as rewarding as industrial research. Given enough time and money, scientists would be able to develop a very high yielding and profitable agriculture. Feeding a fast growing world population would become possible and farming would become as remunerative as industry.

However, this early enthusiasm concealed the fact that with the massive switch to new higher-yielding varieties the traditional and wild ones were neglected, almost forgotten. They were about to disappear, and with them a large proportion of mankind's heritage of plant genes was vanishing.

Emergency collection and conservation of propagating material of the more endangered varieties was undertaken, but by the time the 21st FAO Conference met in 1981 a number of problems remained.

The suggested international convention would contribute to their solution. Its purpose would be to promote the full and free availability of plant genetic resources of agricultural interest for the benefit of all human beings and to establish an international arrangement for co-operation in the collection, conservation and exchange of such resources.

Every nation's sovereignty over its own natural resources is recognized by UN General Assembly Resoultion 1803 (XVII). However, there seems to be a strong international consensus that plant genetic resources should not be subject to any restrictions limiting their availability or exchange for the benefit of agriculture, especially food production. The suggested convention would thus prohibit the imposition of restrictions while safeguarding the rights of states to stipulate reasonable conditions for their export.

But a convention cannot directly ensure the availability of genetic resources under the control of private corporations. Governments could at most try to push through their legislative bodies national laws to apply the principles of the convention to such privately held resources. Besides, since many of the resources subject to restrictions are related to new plant varieties created by private breeders, the proposed convention has to take into account the existence of plant breeders' legislation in some countries.

The new convention would also have to provide the legal framework for the activities now being carried out by the International Board for Plant Genetic Resources (IBPGR) for the collection, conservation and exchange of germ plasm. It is proposed that the international community as a whole assume responsibility for the continued maintenance of the network, perhaps expanded, of institutions co-ordinated rather informally by the IBPGR. This network consists of 35 institutions in 28 countries that give top priority to the collection and conservation of 50 crops. All these institutions have gene banks; they conserve triplicate collections of germ plasm, including "base collections" of 33 crops.

An international gene bank under the auspices of FAO would complete the proposed set up. Advocates envision such a bank as one of the constitutional components - the convention and the network of national banks would be the other - of a global system that would be to plant natural resources what the Law of the Sea is to marine resources: a statute recognizing both national ownership of the plant resources within each country's borders and international entitlement to them as part of the heritage of mankind as a whole. It would also regulate exchanges, including the exchange of technology for resources.

The establishment of this gene bank within the framework of the United Nations system is a key part of the proposal, but is resisted by many who argue that it would be costly and unnecessary. Were it to be an alternative to the IBPGR network it would, in fact, raise considerable problems, surveyed in the FAO Secretariat's paper: conservation of "base" and "active" collections of all kinds of propagating material - not only seeds, their replication and rejuvenation, etc. The cost of establishing and running such a gene bank would be high - almost $6 million initially plus $2.5 a year permanently to conserve a base collection of one million accessions and an active collection of 200,000 accessions, without including the cost of the collection itself.

Supporters of the gene bank maintain that its set up could be considerably simpler and cheaper. Its essential and perhaps only function, they say, should be to conserve under international sovereignty a collection consisting of one of the duplicates of the material in the signatories' own gene banks, to make sure that it would remain freely available even if national laws change or if a country repudiates the convention. Further economies could be achieved by conserving only seeds and not other kinds of propagative material. Most staple food crops would thus remain covered.

The FAO paper concludes: "The figures draw attention to the economic and practical advantages of a system based on the development of a global network of gene banks for which the costs are met for the most part by host countries." (Source: Ceres, No. 94, July/August 1983.)

Despite many critics, protection expands for plant breeders' rights

"Plant breeders' rights" legislation has become one of the most controversial issues of recent years in international agriculture. Proponents argue that patent-like protection to breeders is an incentive to research and promotes agricultural development. Some countries have already adopted, or are about to adopt, laws granting the breeding organizations of new varieties exclusive rights for their commercial use. A few of these countries are grouped into an international organization that is promoting an international convention for the protection of new varieties of plants.

Opponents - and some of them object to even the name - fear that it will reinforce the trend to varietal uniformity and lead to monopolies in the seed trade. It would eventually hinder, rather than promote, development, they say.

Agricultural research in most countries was until recently left in the hands of government agencies, rarely generously funded. However, over the last 50 years achievements of these plant breeders, such as the hybridization of corn and the development of higher-yielding grain varieties, helped to change the prospect.

Plant breeding research had also been carried on in some countries by relatively small commercial seed companies. With varieties - mostly of vegetal and ornamental crops - developed by their own breeders these companies used to compete keenly for the farmers' attention. Fifty years ago in Western Europe seeds from so many so-called improved varieties were being offered for sale, that governments were obliged to establish the first controls ever in the seed trade: licensing systems to protect the farming community from abuse. Only seeds of varieties accepted in the "national lists" were allowed in the market.

In spite of the fact that the licensing systems also gave some measure of protection to the "inventor" of new varieties, the fact remained that in the otherwise unregulated market plant breeding was not financially as rewarding as research had become in other sectors of industry. It was also very costly.

Since governments had difficulty in obtaining from legislatures increased appropriations for long-term research, some agronomists felt that the field should be made attractive to the private sector. A climate generally favourable to legislation granting patent-like rights to the developers of new crop varieties grew out of that situation.

In 1961 France, the Federal Republic of Germany, the Netherlands, Belgium and Italy established the International Union for the Protection of New Varieties of Plants (UPOV) as part of the Geneva-based World Intellectual Property Organization (WIPO), the United Nations agency that deals with patents. Many other countries joined later on or are about to join. UPOV administers an "International Convention for the Protection of New Varieties of Plants", according to which new member countries are to adopt legislation allowing a monopoly of about 20 years for the production and marketing of seed to the breeders of "unique", "uniform" and "stable" varieties. Some countries, like the USA and Japan, which did not join UPOV, have adopted similar patenting systems on their own. Third World countries are slowly following their example and the potential exclusive market for the varieties protected by "plant breeders rights" is still expanding. Research has become attractive for the private sector.

Under the UPOV Convention, to be entitled to Plants Breeders' Rights, a variety must be uniform, clearly identifiable and distinct from others, a demand that leaves no room at all for the diversity so dear to plant breeders. In the European Economic Community national lists were consolidated in 1980 into a Common Catalogue. Since for every entry in the Catalogue high fees are charged, only the most promising are registered, the traditional ones being left out.

The trend to varietal uniformity and the spread of Plant Breeders' Rights Legislation made plant breeding research and the seed industry as a whole amply attractive to big business. During the last 10 or 15 years a number of transnational corporations have entered the field, many through the acquisition of smaller traditional seed firms. Some consider this a welcome addition of capital resources and management ability to the perennially neglected sectors of agricultural research and development. Opponents fear that they may come to monopolize the whole field of agricultural inputs and eventually hinder, rather than further, the world's progress in agricultural matters. (Source: Ceres, No. 94, July/August 1983.)

US National Institutes of Health involved in law suit

In what is being touted as a major legal test for genetic engineering, several groups recently filed suit in the U.S. District Court in Washington to keep the National Institutes of Health from permitting the release of gene-spliced substances from the laboratory. The suit is the first legal challenge to the use of engineered organisms outside a laboratory. Other plaintiffs are Environmental Action and Environmental Task Force, both based in Washington. "A careful examination of the internal operation of the NIH Recombinant DNA Advisory Committee demonstrates a disregard for both the spirit and the letter of the National Environmental Policy Act," say the plaintiffs.

NIH is still studying the suit and is not expected to formally respond for some time, but a spokesman for NIH says that the agency and its Recombinant DNA Advisory Committee (RAC) have routinely been guided by the Agriculture Department and by outside experts.

The suit fuels an already intense issue in Washington. In Congress, a House subcommittee chaired by Representative Albert Gore, Jr., (D., Tenn.), is investigating the potential risks of release, and federal regulatory agencies, including the Environmental Protection Agency, have begun studies of their own.

So far, NIH has approved three release projects. The agency has authorized a Stanford University researcher to field-test genetically modified corn plants, a Cornell University researcher to test genetically engineered tomato and tobacco plants, and two researchers at the University of California at Berkeley to employ genetically engineered bacteria to control frost damage in plants.

In their suit, the plaintiffs contend that NIH has failed "to establish adequate scientific protocols and for evaluating the environmental risk of such experiments." Officials of the Foundation on Economic Trends insists that "many of the appropriate methodologies and testing procedures do not yet exist to assess the risk factors" of release, and will take several years to develop. Further, the foundation argues that RAC lacks the scientific expertise to assess such risks. "There are," it notes, "no ecologists, botanists, plant pathologists or population geneticists on the committee." The groups' concerns are shared by several ecologists. (Extracted from Chemical Week, 21 September 1983.)

EPA decides to regulate new biotechnology products

The Environmental Protection Agency (EPA) has decided that it has the authority to regulate new biotechnology under the Toxic Substances Control Act (TSCA). This authority may be challenged, but only through costly litigation. Meanwhile, the EPA expects to have $3 million in its budget next year and about 20 people to monitor biotechnology companies' work.

Until now the Federal Government has depended upon companies' voluntary compliance with guidelines of the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health for research and manufacturing safety. Agencies such as Food and Drug Administration or Department of Agriculture regulate the products. EPA generally tries not to overlap what other regulatory agencies are doing but the agency bas a great deal of latitude. The EPA is probably not primarily concerned with manufacture of genetically engineered products but with recombinant DNA organisms that might be released into the environment when used for oil recovery, metal ore leaching or waste disposal. Genetically engineered higher plants might also come under scrutiny, but EPA's attitude is far from clear.

Some of IBA's member companies are thinking of challengine EPA's authority in the courts. However, IBA members are predominantly in favor of working with EPA to establish a set of reasonable regulations the industry can live with. (Source: Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

US public invests nearly half billion $ in genetic engineering

If the three latest stock offerings planned by genetic engineering companies are successful, public investment in 23 genetic technology firms via U.S. underwriters will total about $450 million for the past five months. Total does not include private placements or R&D limited partnerships. Here are the latest market entries:

1. Advanced Genetic Sciences (AGS)
2. International Genetic Engineering (Ingene)
3. Bio-Technology General (BTG)

Estimated Spending for Genetic Engineering R&D
Performed at U.S. Commercial Facilities in 1982

Type of Firm	Spending
Genetic Engineering, Publicly Held	$110 million
Genetic Engineering, Privately Held	40
Other Companies	150
Total	$300 million

For 1983, the figure will be higher. As products move from the laboratory into the pilot plant, costs go up several fold. An increase of 15% to 20% is a reasonable estimate for 1983.

(Source: Genetic Technology News, August and September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Biotechnologists may turn to AI (Artificial Intelligence)

Biotechnologists are finding that a new generation of computer programmes are now able to provide answers to many problems and through Artificial Intelligence (AI). Essentially, AI is a way of transforming a computer into a problem-solving tool through the use of what its practitioners call "expert systems." Computers are programmed to mimic both the reasoning and intuition of human experts, to emulate the way people use past experience to tackle new problems. Such systems can weigh facts and assumptions and make choices because they are not trapped in any specific decision path.

A Palo Alto AI firm, IntelliGenetics, is introducing programmes to optimize fermentation scale-up - a major bugbear of biotechnology.

Successful scale-up depends on establishing and sustaining just the right biochemical environment, notably such variables as temperature, salt concentration, nutrient mix, pH, and in particular the idiosyncrasies of each individual microorganism. AI can interactively control this unsteady-state fermentation process. At points where the human user doesn't agree with the machine's strategy, the programme's knowledge base can be edited and thus be continuously tuned.

Another potential for Artificial Intelligence is the large-scale culture of plant cells. Present methods of plant tissue culture are largely empirical and an Artificial Intelligence approach could codify the aspects into reproducible technology, making it available to a wider circle of users. Artificial Intelligence is being applied to extend the genetic engineer's reach from linear sequencing to tertiary modeling. Such expert systems will recommend protein structures to perform desired functions, work out the gene sequence needed to produce the protein, advise on gene insertion, and product recovery. Conceptually, Artificial Intelligence will go on to optimize production of the new substance by scale-up of the appropriate fermentation system.

IntelliGenetics is offering Artificial Intelligence programmes for large-scale DNA and amino-acid sequencing, restriction maps from enzymatic digests, and simulation of recombinant-DNA experiments. These silicon-chip tools are being used by biotechnology R&D firms such as Amgen of Thousand Oaks, Calif., to plan the engineering of host organisms.

Geneticist Frederick R. Blattner of the University of Wisconsin, Madison, has begun marketing some three dozen Artificial Intelligence programmes that analyze comprehensive nucleic-acid-sequence data. Gene-splicing programmes are also being offered by Dr. Roger Staden of Britain's Medical Research Council in Cambridge, England and Japan's Ministry of International Trade and Industry (MITI) plans to spend half a billion dollars over the next decade to develop an advanced computer targeted specifically at Artificial Intelligence applications, including biotechnology. (Extracted from McGraw-Hill's Biotechnology Newswatch, 6 September 1983.)

Uranium extraction

Microbes may interfere with in-situ extraction of uranium, according to the New Mexico Institute of Mining and Technology (Socorro). The Institute, which performed a study for the U.S. Bureau of Mines, found high concentrations of soil bacteria in four ore samples from mining operations that had experienced plugging problems underground. In subsequent laboratory tests, microbes proliferated in a simulated underground environment and decreased the permeability of uranium ore samples by an order of magnitude in 20 days.

In-situ extraction is done by injecting a leaching solution into an orebody, then recovering the solution and dissolved minerals from a well. The bacteria are apparently introduced into the orebody by the drilling and injection process, and once there they thrive under the leaching conditions. The leach solution is usually a fairly neutral mix of carbonate/bicarbonate, with an oxidizing agent (typically hydrogen peroxide) to convert the uranium's +4 valence to +6, so that it will form uranyl carbonate. However, the oxygen present also promotes bacterial growth.

A possibly remedy may be to inject stronger concentrations of peroxide into the well to break down the organisms. Some companies have tried this with some success, says the Institute, but it has to be repeated periodically. (Source: Chemical Engineering, 11 July 1983.)

EEC can turn biomass into profit

EEC countries could produce energy equivalent to 2 million b/d of oil from biomass and show a profit, according to Professor David Hall of the University of London.

There are 86 million tonnes of oil equivalent (TOE) of net energy available from human and animal wastes, wood and energy crops and a further 22 million toe could be recovered from agricultural surpluses. (50 million toe is the equivalent of 1 million b/d.) These resources could provide feedstock for up to 16,000 conversion plants processing 25 tonnes/day of biomass. The average estimated cost of a plant would be about ECU130,000 ($75,400).

The processes would produce charcoal, bio-oil and bio-gas worth ECU870 million a year, calculated at current market prices for fuels of comparable calorific value. The amortized cost of the investment plus salaries and the price of the feedstock would total ECU829.6 million a year, giving a profit of ECU40.4 million a year. The factories would create 430,000 jobs.

Current community surpluses of beef and dairy products suggest that there are 2.5-3.5 million excess cows on the land. These are subsidized at a rate of £120 ($189) an animal each year. If the cows were eliminated, 1.2-1.7 million hectares of land would be freed for cultivation of energy crops and the £420 million saved in subsidy. This could be invested in their development.

The argument that massive use of biomass could lead to a lack of food is invalid, he says. At current production rates, surplus grain production will total 10 million tonnes/year by the end of the decade. The problem is how best to balance food and fuel production.

According to the calculations, biomass could provide up to 7 per cent of all EEC energy needs by the end of the century.

Total net (after conversion) energy potential from biomass in EEC countries (millions of tonnes of oil equivalent)

Wastes	
Animal wastes	11.5
Crop residues	12.5
Forestry/wood residues	7.9
Municipal solid wastes	8.9
Sewage	1.8
Firewood	6.6
Crops	
Forestry plantations	28.1
Catch crops, algae and aquatic plants	8.5
Surpluses*	
Sugar	2.6
Fruit withheld	0.7
Fruit losses	1.6
Potatoes	8.5
Cereals	8.6
Total	107.8

* Other potential surplus resources include 1.84 gallons of wine and the crops which could be grown on 1.7m hectares of land freed if dairy cattle production were not in surplus.

(Source: World Solar Markets, July 1983.)

Some biotechnology companies beginning to generate profits

(Dollar amounts are in millions)	Sales		Earnings		Profit Margin	
	Latest period	Change from 1982	Latest period	Change from 1982	Latest period	Year-ago period
Three-month results						
Bio-Rad	$16.4	+26%	$0.185	NM	1.1%	-2.8%
Centocor	1.6	+242	-0.269	NM	-16.8	-180.6
Collaborative Research	1.0	-45	-1.725	NM	-176.5	-6.0
ens Bio Logicals	0.1	-62	-0.888	NM	-616.7	-357.1
Enzo Biochem	1.0	+310	0.238	NM	24.5	-138.0
Genentech	11.0	+44	0.332	+310	3.0	1.1
Genex	1.9	+8	-1.800	NM	-95.1	-73.9
Microbio. Sciences	4.3	+17	0.212	+114	4.9	2.7
Ribi Immunochem	0.2	+122	-0.084	NM	-51.2	-110.8
TechAmerica	10.0	+28	0.172	NM	1.7	-1.5
Totals	47.5	+29	-3.626	NM	-7.6	-12.9
Six-month results						
Novo Industri	179.2	+19	39.074	+58	21.8	16.5
Fiscal-year results						
Cetus	28.9	-12	-4.671	NM	-16.2	9.2
Collagen	12.4	+68	1.293	+85	10.4	9.4

Results are through June 30 except for Collaborative Research, whose fiscal quarter ended May 28. (NM) Not meaningful.

(Source: Chemical Week, 14 September 1983.)

RECENT DEVELOPMENTS

Co-transformation

A method has been patented to introduce foreign genes into animal cells in laboratory culture so that the transformed cells can be recognized. The technique could be useful in production of interferon, blood clotting factors, hormones, vaccines, etc., and in developing a successful gene replacement therapy. The patent, awarded to R Axel and S. Silverstein of Columbia University and M. Wigler of Cold Spring Harbor (NY) Laboratories, covers the technique called co-transformation, which inserts two unrelated genes simultaneously into cells. Animal cells growing in culture could be used to produce biological substances now difficult or impossible to make in bacteria. Patent applications are also pending in Canada, Europe, Japan and Australia. (Source: Technology Update, 24 September 1983.)

Calcitonin gene has two faces

Molecular biologists and neurophysiologists at the University of California, San Diego, have now combined their expertise and demonstrated a surprising example of the versatility of genes that carry the code for chemical communication. Michael Rosenfeld and colleagues have discovered a gene which makes a hormone in gland cells, but an entirely different and hitherto unknown product in brain cells. Predictions from its structure show that the gene's mysterious alter ego is the precursor to a new molecule which may be involved in relaying hot, cold and painful sensations in the nervous system, and in the control of blood pressure.

The hormone calcitonin is made in the C-cells of the thyroid gland and is an example of a chemical messenger with an important body-wide function. Its principal targets are bones and kidneys, where it controls the levels of calcium in extracellular body fluids. Three years ago, scientists at San Diego used a thyroid tumour which produced large quantitites of calcitonin to generate and clone part of the DNA sequence of the gene from which this hormone originates. By repeating this process they were able to deduce the molecular structure of the entire gene. It turns out that the calcitonin gene sequence contains several exons - the parts of the DNA which can potentially be transcribed to messenger RNA - between the introns (or untranscribed DNA portions). This is a common feature of many genes but normally, all the exons and introns in the DNA sequence appear joined together without a break in the RNA transcript. In the thyroid tumour cells, however, one out of the four exons present in the gene was not transcribed. Scrutiny of messenger RNA produced by the tumour cells showed that the silent exon was transcribed instead of that encoding calcitonin. It seemed unusual that the missing sequence of the gene should only be expressed during the freak behaviour of thyroid cancer cells. If the product of the silent exon was to be of any use, conditions must exist where it would be produced under more stable conditions.

The DNA code of the silent exon was translated by computer into the structure of the molecule it would hypothetically produce. Secondly, this hypothetical hormone was prepared artificially using an automatic synthesiser. Thirdly, the synthetic molecule was injected into a rabbit which produced antibodies against it. Finally, the rabbit's immune serum was withdrawn and used to detect the possible presence of the new messenger molecule in tissues throughout the body.

The results, as expected, showed that the hormone encoded by the silent exon was undetectable in normal thyroid tissue. But far from expectations, the new messenger molecule (now called calcitonin gene-related peptide or CGRP), was distributed widely among cells in the nervous system of the brain and peripheral organs. Abundant quantities of the gene were found in the small neurones of the sensory ganglia. These cells are involved in conveying sensory information on temperature and pain to the perceptive centres of the brain. These neurones may use CGRP to communicate with other parts of the nervous system, passing impulses in sensory pathways to their final destination, the cerebral cortex. (Source: New Scientist, 18 August 1983.)

Bacterium yields insight into photosynthesis

Plant genetic engineers could probably do more to enhance output of crop or forest plants by increasing plant photosynthetic efficiency than in any other way, but before they can start shifting genes to improve photosynthesis they must first learn how the process works. This will take a while, but a research team of University of California's Lawrence Berkeley Laboratory has just added some knowledge that will help. They have identified five genes which control the first steps of photosynthesis in the bacterium Rhodopseudomonas capsulata. Three of the genes code for proteins that make up the reaction centre, where light energy absorbed by chlorophyll and other pigments separates positive and negative

electrical charges within the thylakoid membrane. The reaction centres are part of the intricate thylakoid membrane, where photosynthesis takes place. The separated electrical charges operating with the membrane provide energy to drive the complex chemical reactions involved in photosynthesis.

The Lawrence Berkeley team isolated the genes by breaking down a large, 50,000-base pair (bp) segment of DNA contained in the R' plasmid of the bacterium. The segment supports photosynthesis when inserted into bacteria. The group used restriction enzymes to obtain smaller fragments of the large DNA segment which were cloned in Escherichia coli to obtain a large number of copies of each fragment. These were then used to transform R. capsulata. Colonies of transformed bacteria containing the fragments with photosynthetic genes could be spotted because by fluoresced under light. The three reaction centre genes and two genes with other functions in photosynthesis were picked out of the much larger DNA segment in this way. The DNA sequence of one of the reaction centre genes was compared with DNA from chloroplasts from spinach and tobacco plants. Comparison showed DNA sequences have similarities. This indicates that the photosynthetic process in R. capsulata is probably fundamentally the same as that in higher plants. (Extracted from Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

First artificial chromosome built

Two workers at Harvard Medical School, Andrew Murray and Jack Szostak, have constructed the world's first working artificial chromosome. Yeast cells copy the chromosome faithfully and pass the copies on to their daughter cells when they divide. The work holds out the hope of more effective therapy for inherited diseases, although this was still a few years off.

Chromosomes are the packages of DNA and associated proteins that carry the genetic message inside the cell nucleus. They are composed of at least four distinct functional parts: the genes, the replication origin, the telomeres and the centromere. Murray and Szostak used the full battery of tools available to the genetic engineer to assemble these into their chromosome.

The heart is the centromere, which is responsible for attaching the chromosome to the spindle of the dividing cell. The spindle ensures that, after division, each daughter cell gets one copy of the newly duplicated chromosome. Murray and Szostak were given their centromeres by Lousie Clarke and John Carbon at the University of California in San Diego. Clarke and Carbon have isolated and cloned the centromeres from several yeast chromosomes and, in some cases have determined the full DNA sequence.

To one of these centromeres Murray and Szostak added a replication origin, a sequence that enables a length of DNA to be copied by the cell's machinery. The genes they chose to put on the artificial chromosome enabled the cell to manufacture its own leucine and uracil. These are important substances that have to be supplied ready-made to the recipient yeast cells. Any cell that took up a working copy of the artificial chromosome would be able to make its own leucine and uracil, and can be easily identified because it will grow in a medium without these substances.

Finally, Murray and Szostak added telomeres - the special sequences at the ends of chromosomes - snipped from a protozoan called Tetrahymena. The resultant chromosome was copied and passed to daughter cells, but was not very stable. It was about 10 times shorter than natural yeast chromosomes, a difference that turned out to be important. Murray and Szostak inserted their chromosome into a much longer piece of DNA and got a new artificial chromosome, about 55,000 bases long, that was less stable than the real thing, but performed very well. (Extracted from New Scientist, 22 September 1983.)

Dental cavities might be filled with natural enamel made by rDNA

Some day dentists may be able to use a natural dental enamel rather than the metals or polymers now used to restore teeth. Genes coding for two proteins in mouse tooth enamel - which is analogous to human enamel - have been cloned in Escherichia coli by workers at University of Southern California.

Cloning enamel genes is an off-shoot of a programme, started many years ago, that aimed to learn how body cells differentiate into teeth during embryonic development. In 1980 Dr. Slavkin turned to recombinant DNA as a tool to study the genes involved. The possibility of developing a natural restorative material grew out of this work.

Dental enamel, like bone or mollusc shells, consists of a protein matrix that forms outside of living cells. The protein contains nucleating points at which inorganic crystals can form and grow. The end result in tooth enamel is a material that is 99 per cent inorganic. The crystals, of a calcium phosphate, found in teeth are very large by biological standards although they are microscopic. Dental proteins forming the matrix fall into two classes, enamelins and amelogenins and the genes that code for a representative of each class have been cloned but so far has not been possible to use the proteins produced in E. coli to form a matrix upon which the calcium phosphate crystals can grow. Part of the difficulty in doing this arises from the fact that E. coli and other bacterial proteins are not glycosylated or phosphorylated, as natural dental proteins are.

It is intended not to try to produce recombinant microorganisms but rather that could be placed in a cavity to produce natural enamel in place to be able to develop a system to mix tooth proteins and an inorganic to form the restorative. (Extracted from Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, NJ 07024, USA.)

Split genes now found in bacteria

Most genes of the higher organisms contain stretches of DNA that are not used to make the final protein or RNA product of the gene. These apparently redundant regions (called introns) are found inserted between the functional parts of a gene, and once the gene has been copied into RNA they are soon removed by "splicing". The discovery that the genes of higher organisms are split in this way provided molecular biologists with one of their biggest surprises in recent years. A team of American scientists has now found evidence for split genes in bacteria as well, suggesting that the phenomenon might be much more widespread in nature than had previously been thought.

Up till now attempts to find split genes in bacteria have always been successful. But the bacteria can be separated into two distinct groups, the eubacteria and the archae-bacteria, and all of the preivous searches were confined to the eubacteria. Carl Woese, Brian Kaine and Ramesh Gupta of Illinois University decided to take a look at the archae-bacteria, and in a bacterium called Sulfolobus solfataricus they found what they were looking for - two genes that appear to contain introns.

The genes concerned are not actually used to make protein. Instead, they code for transfer RNA molecules, which play a vital role in protein manufacture. The important point is that the initial RNA copy of the genes will contain unwanted portions of RNA that will have to be removed by splicing.

If split genes turn out to be quite common in the archae-bacteria then it might help scientists to investigate the precise mechanisms of splicing and the reasons why split genes occur in the first place.

So far no real function has been found for introns, but they may be involved in regulating gene activity in some way. Another interesting idea is that constructing proteins from split genes might allow a greater flexibility in protein evolution. (Source: New Scientist, 14 August 1983.)

Gene therapy closer

Efforts to implant a missing gene in a person born with an enzyme-deficiency disordr took a long stride toward realization this summer. Two research groups at San Diego, Calif. teamed up to try retroviruses as a human-gene transfer vehicle for restoring enzyme synthesis in the cultured cells of patients with Lesch-Nyhan syndrome.

At the Salk Institute virologists Inder M. Verma and A. Dusty Miller decided to use retroviruses as cloning vehicles. Together with pediatric geneticists Douglas J. Jolly and Theodore Friedmann of the University of California at San Diego (UCSD) an RNA virus was inserted with the DNA sequence coding for hypoxanthine-guanine phosphoribosyl transferase (HPRT), the enzyme that Lesch-Nyhan victums cannot make. Some of their protein-coating genes were stripped from these retroviral vectors to make room for the HPRT sequences and the new vector was leaned up with "helper" retroviruses to help them replicate. The cloning package not only entered human Lesch-Nyhan cells in vitro but expressed the HPRT activity they laked with high efficiency - at the level of normal cells.

Lesch-Nyhan syndrome is one of the human diseases caused by an abnormality in a single gene. The HPRT enzyme that its victim's cells cannot synthesize is part of a metabolic salvage pathway in which purines are recycled into nucleic acid rather than beir degraded to uric acid. The sex-linked disorder afflicts only males; some months after birth the

seemingly normal Lesch-Nyhan infant shows mental retardation, spasticity, and a trait of self-multilation. Victims usually die in their teens or twenties. (Extracted from McGraw-Hill's Biotechnology Newswatch, 17 October 1983.)

The new age of hormones

Dr. Terence Chadwick of Novo Laboratories, Hampshire, described the beginning of an era in which it will be possible to manufacture at will, hormones that largely determine physical and temperamental idiosyncracies as well as others not yet thought of, in quantity, and precisely tailored to human acceptance.

A look at human growth hormone will show the state of the art. Human growth hormone influences stature. If you have too much you are a giant; but an estimated one in 5,000 children may suffer from deficiency and, deficiency may account for a half to two-thirds of all children who are referred to hospital because of short stature. Deficiency is corrected by giving more; but growth hormone works in humans only if taken from other humans, so the source is confined to pea-sized pituitary glands from human corpses, and supplies are expensive and strictly limited.

The hormone is a polypeptide (a small protein); with 191 amino acids, and the entire molecule can now be made by genetic engineering. Indeed, its production involves and illustrates the two principal techniques that genetic engineers now employ. The first, used to put together amino acids 25 to 191 in this particular hormone, is the DNA copy technique. First the RNA that produces the required amino acid sequence is isolated from cells that contain a lot of it; in this case cells of the pituitary. Then that RNA is induced to create a DNA facsimile of itself - exactly reversing the process whereby DNA normally produces RNA in the cell, and indeed using enzymes that work backwards. Then the RNA is stripped from the DNA it has made, and the remaining single-strand DNA replicates itself to produce a double-stranded length of DNA - which indeed is effectively a gene. The DNA is then carried by appropriate vectors into bacteria, which multiply (clone). Inside its new bacterial hosts, the newly constructed gene produces more RNA that in turn produces the required polypeptide.

The second technique involves the "gene machine", in which appropriate DNA is built up step by step from its constituent nucleotides. First two nucleotides are put together in a column of resin beads (which provide a convenient surface for reaction); and when they have joined, the surplus is washed away; the next nucleotide is introduced, and so on until the required nucleotide sequence is built up, which will code for the required amino acid sequence. It is by this method that the amino acid sequence 1 to 24 has been provided for human growth hormone. After growth hormone, synthesis and application of the newly identified growth hormone releasing factor may be contemplated, deficiency of which is another cause of short stature. Then again, growth hormone itself might be used to increase the height of women with Turner's syndrome (caused by a defect of the female sex chromosomes). It has been suggested that such treatment would help in Turner's syndrome, but no one has had enough spare growth hormone to put it to the test.

Indeed, the general point is not that the body's many polypeptide hormones have not hitherto been available at all (if that were the case one would hardly know of their existence) but that supplies have been so limited that no on has been able to test the effects of giving more or less of any particular hormone to this person or that, as has been possible with drugs. Yet the potential of endocrinological intervention is very evident. (Source: New Scientist, 25 August 1983.)

Toxic shock syndrome

The toxin that causes toxic shock syndrome, a disease associated with the use of high-absorbency tampons, has been isolated and identified by a microbiologist at the University of Wisconsin in Madison. Merlin Bergdoll reported in the journal Biochemistry that the toxin, produced by Staphyloccus bacteria, is closely related to one that causes a common form of food poisoning. Using the toxin, Bergdoll has come up with an assay system that tampon manufacturers could use to study how certain materials stimulate the toxin's production and to screen out those tampon materials that are potentially dangerous. Bergdoll is now growing antibodies to the toxin. His goal is the development of a clinically useful diagnostic test. (Source: Chemical Engineering, 22 August 1983.)

New method of cancer detection

Improved prospects for patient recovery should result from a new cancer detection method marketed by Labsystems Oy, of Helsinki. The system is based on research conducted at the City's univrsity, and it determines the origin of different types of tumours quickly and

reliably. The correct diagnosis of the type of tumour is the decisive factor in choosing therapy, so the new technique enables the correct course of treatment to begin promptly, enhancing recovery rates.

The new method uses a chemical marker in conjunction with mono-clonal antibodies to identify the type of cell from which the tumour originated, even from a secondary tumour. Labsystems say that tests can be completed in 30 minutes using standard pathology laboratory equipment. (Source: The Hindu, 27 July 1983.)

Researchers find test for breast cancer

A test originally develped to detect cervical cancers has been found to work for breast cancer, according to a recent report. The test can pick out malignant cells long before they begin to look abnormal, and so raises hopes that earlier treatment could stop many deaths among women who contract breast cancer every year.

The test was developed by Dr. Andrew Sincock and Dr. Jeff Middleton at Queen Elizabeth College in London, and funded by a charity, Quest for a Test for Cancer. In normal growing cells, DNA is tightly bound to its associated proteins. Before the cell divides, the DNA must free itself of some of these proteins. Because cancer cells are "trapped" in an endless cycle of growth, their DNA is always in the "free" state, and it is this free DNA that the cancer test detects. By taking DNA from breast tumour cells and attacking it with dilute acid, the DNA is chemically changed so that it takes up a purple stain known as Schiff's reagent.

It was found that malignant cells take up more than three times the amount of dye than normal cells. A machine called a microdensitometer then measures the dye bound to the DNA in nuclei - but the technique works so well that even the naked eye can see the difference.

The correlation between the amount of free DNA in cells (and so the density of stain), and the degree of malignancy is so good that Sincock envisages the technique eventually being able to grade the malignancy of tumours. The immediate use in breast and cervical cancers, though, is to allow doctors to predict which patients will go on to develop aggressive tumours, and so tailor the treatment accordingly. (Source: New Scientist, 4 August 1983.)

Oncogene researchers may run cancer risk

Researchers working with cancer-causing viruses could run the risk of getting cancer themselves. Three Australian workers claim that splicing cancer genes into the bacterium Escherichia coli, which is commonly used in the laboratory, poses a hazard research workers either do not recognize or ignore.

Ditta Bartels, Hiroto Naora and Atuhiro Sibatani, who work at the University of Sydney, the Australian National University, and the Commonwealth Scientific and Industrial Research Organisation, argue that instead of performing such work under the minimum safety requirements of "good laboratory practice" - carried out openly on the laboratory bench - they should be done with protective clothing, enclosed cabinets, special fume cupboards, and other measures for greater safety.

The confidence of scientists working in this field of research rests on work done in 1978, with a polyoma virus. It involved breaking up the circular virus with restriction enzymes. They stitched the fragments of the virus's DNA into the genetic material of E. coli and found that the virus' infectivity was lost when injected into mice.

Scientists assumed that the genetically engineered virus was less harmful than the original because it had been chopped up. They thought that by enclosing the virus in E. coli's DNA, they had somehow changed its properties. Consequently, these experiments are generally done without special safeguards.

The three Australians say that, today, most research on cancer-causing viruses involves another type of virus which is not circular but linear. These "retroviruses" do not need to be chewed up so much by restriction enzymes, so more of the intrinsic order of DNA remains intact. These viruses could, theoretically, readily replicate in the hosts cells, and move out into the body, like an ordinary virus.

Eventually, one of these viruses carrying a lethal oncogene might reach a susceptible cell and trigger off the growth of a tumour. The oncogene might be "dormant" but Dittels thinks the very act of cloning in bacteria activates the oncogene. (Source: New Scientist, 25 August 1983.)

Cytogen will develop cancer drug delivery system

Outlook for a marketable monoclonal antibody/chemotherapeutic agent against cancer moves a step closer. Cytogen will use its proprietary technique for antibody modification to develop a cancer drug delivery system for American Cyanamid (Wayne, NJ), which has just purchased 15 per cent of the company for $6.75 million. Cytogen's technique can be used to attach methotrexate, a major cancer chemotherapeutic marketed by Cyanamid's Lederle Laboratories (Pearl River, NY) to antibodies that will bind specifically to cancer cells. This should make the drug more effective by placing it exactly where it is needed. At the same time side effects that result from circulation of the drug throughout the body for long periods should be reduced. Monoclonal antibodies have been developed that are specific for various types of cancer cells, but by no means for all types. Cytogen's technique makes use of a proprietary co-valent linker to connect drug and antibody. The linker is conditionally unstable, so it can be made to release the drug when wanted, or the drug may be left attached. (Source: Genetic Technology News, July 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Two genetic changes necessary to transform normal cells into cancerous ones

US and UK researchers discovered that at least two individual genetic changes are normally needed to transform normal cells into cancerous cells, showing why it takes years for cancers to develop. New research at MIT, Cold Spring Harbor, and the Institute of Cancer Research (UK) supports the existing understanding of cancer researchers that the development of cancer at the cellular level occurs in stages. These two processes will push a typical cell perhaps 90 per cent of the way to being a cancer cell. The findings are crucial partly because they give an explanation in molecular terms of the long-held observation that many types of cancer required at least two steps, usually with a long interval between them. (Source: Technology Update, 3 September 1983.)

Defective collagen gene kills embryos

Rudolph Jaenisch and his collaborators at the University of Hamburg last year reported a breakthrough in the study of mammalian development when they succeeded in deliberately creating a mutation which affected the development of mouse embryos. The mutation was created by a retro-virus which inserted itself into the animal's DNA and prevented the activation of a specific gene about halfway through the mouse's development, causing it to stop developing and die.

With Angelika Schnieke and Klaus Habers, Jaenisch has now gone on to establish that the gene whose inactivation has this lethal effect is one of the genes that codes for collagen - just about the commonest molecule in the body. Collagen defects can prove lethal to human babies soon after birth, so this research could be far-reaching. Jaenisch's technique for making mutant mice is to inject large doses of retroviral DNA into early mouse embryos. The DNA becomes inserted at random into various sites in the embryo's chromosomes, and some of the inserted DNA is passed on to future generations of mice. The lethal mutation occurred in a strain of mice known as Mov-13. Jaenisch was able to breed these mice because the mutation is lethal only if inherited from both parents: if the mouse has one normal gene it can survive and breed.

Jaenisch was able to locate the inserted DNA, and thus pinpoint the site of the affected gene, by using radio-labelled copies of the viral DNA. Also, he showed that the gene is turned on in normal embryos, but not in Mov-13 embryos, at about the stage when the Mov-13 animals stop developing. More experiments with radio-labelled copies of the gene itself (rather than the viral DNA inserted in it) showed that it is normally active in certain cells of skin, bone and muscle, and that it tends to be switched off in cells that have become cancerous. Two other important facts helped Jaenisch and his colleagues to guess what the gene might be. First, a virtually identical gene proved to have a virtually identical pattern of activity in tissues from several different species, so it obviously produced a common molecule essential to all animals. Secondly, if cells were extracted from Mov-13 embryos before they died and grown in culture, they survived. So the molecule could not be necessary for the survival of individual cells.

Putting all these facts together, the Hamburg team concluded that the molecule must be one that cells commonly secrete into their environment, to form what is known as the extra-cellular matrix, and the commonest of all is collagen. So the scientists compared a cloned collagen gene with the gene they had identified in Mov-13 embryos, and that was how they found out that the mutation was in the collagen gene.

This leaves the quesiton of why the absence of collagen should kill a mouse embryo at 13 days. Jaenisch and his collaborators guess that collagen may provide a vital clue to an important step in development that normally occurs between the 11th and 14th day. One such step, for example, is the beginning of blood cell production in the embryo's liver. The differentiation of blood cells depends on components of the extracellular matrix secreted by other embryonic cells; collagen may be a crucial component.

Humans with collagen defects usually show only one affected gene; inheriting a normal gene from the other parent. There are various effects on the individual but the commonest is brittle bones. Jaenisch's Mov-13 mouse strain may help to clarify the problems with the human disease. (Source: New Scientist, 11 August 1983.)

Liver cells persuaded to make insulin

The dream of diabetics to throw away their pumps and syringes and once again make insulin naturally may eventually come true, thanks to the researches of a French scientist. Dr. Claude Nicolau's work at the Centre de Biophysique Moleculaire in Orleans brings one step closer the notion of using gene therapy to cure diabetes and maybe even other severely debilitating diseases, such as the inherited blood disorder thalassaemia. Nicolau used a package of genes to activate insulin production in rat livers, an organ which does not normally produce this protein which is essential if the body is to metabolise glucose correctly. Blood-glucose levels in rats dropped by 33 per cent, compared with controls, after the insulin genes were introduced to the liver cells, showing that glucose was being metabolised. Although it is much too early to apply this method to humans, Nicolau's experiments over the next year could bring these essential clinical tests much closer.

Gene therapy generally means either replacing defective genes in the nuclear chromosomes of the cell with others that function normally, or sometimes activating the genes of dormant "immature" cells to manufacture a desired protein. Nicolau's work did not quite achieve this: his package of genes functioned independently in the nucleus of the cell, but nonetheless produced active rat insulin.

All the cells in the body possess the same complement of genes, and theoretically could produce any desired protein, but the genetics of some may be more easily tinkered with than others. Liver cells in particular could be genetically converted into factories for producing a range of human proteins, because there exists already an easy way of getting foreign genes into the cells, which Dr. Nicolau, working with groups in the United States and Israel, tried successfully in their rats.

Nicolau stitched the rat gene for a precursor of insulin (preproinsulin), into a ring of bacterial DNA (a plasmid), and encapsulated both in a liposome. Lipsomes are fat sacs which are currently being tested by drug companies as a means of delivering to the tissues. Six hours after injection into the test-rats, Nicolau compared glucose levels (a measure of the insulin available) with four control groups, which received empty liposomes, or liposomes carrying only a plasmid (without the insulin gene), the free rat insulin gene, or nothing at all. The controls showed an unchanged level of blood glucose, at around 107 mg per 100 ml of blood, whereas in the gene-treated animals, blood glucose stabilized at around 72 mg/100 ml. More spectacularly, the amount of insulin in the livers of test-rats more than doubled, compared with controls.

Nicolau says that in all the gene-treated cases the "prepro" form of insulin was processed to physiologically active insulin, but he admits in these experiments gene "expression" of active insulin stopped 12 hours after innoculation. However in his latest series of tests with a modified liposome system the foreign gene is still working after a month, but it is much too early to try such gene therapy in humans. (Source: New Scientist, 1 September 1983.)

New hepatitis B vaccine developed

A cheap and safe vaccine against hepatitis B, may soon be available, thanks to genetic engineering.

A group of researchers in Holland, collaborating with Professor Kenneth Murray of the University of Edinburgh, announced that its new recombinant DNA vaccine effectively protects chimpanzees against hepatitis B infection. The similarity of the chimpanzee disease to that in humans raises hopes for an effective human vaccine.

The new vaccine holds out the even more exciting promise of preventing one of the most common cancers in man, hepatocellular carcinoma, or primary liver cancer. Most of the 250,000 people that develop the disease each year come from developing countries.

The American pharmaceutical company Merck, Sharp and Dohme already sells a very effective vaccine prepared from the infected blood of American donors. It consists of part of the virus coat, the hepatitis B surface antigen, which provokes the body's immune system to manufacture antibodies against the virus. The disease is not transmitted by this antigen but by the DNA in the viral core. Even with Merck's very pure, WHO-approved vaccine there are fears that the donated blood could pass on other dangerous viruses, perhaps causing diseases such as acquired immune deficiency syndrome (AIDS). Supplies of human blood are limited, and each batch must be tested over many months on chimpanzees - and each animal costs $6,000. Because of this, Merck's product is expensive, costing around $100 a dose.

The strategy adopted by Murray and Dr. Albert Hinnen to obtain large quantities of these proteins cheaply, was to insert the gene which codes for the surface antigen into the genetic material of yeasts. The yeast "host" cells then make the viral protein along with their own. When a team at the TNO Primate Centre at Rijswijk in Holland, under Dr. Huub Schellekens, tested Murray's preparation on four chimpanzees, the scientists found that two animals remained healthy after they were challenged by hepatitis B virus. This is the first report of a successful test with the genetically-engineered product in higher primates, and means that a vaccine suitable for human use is not far away.

Even at a theoretical cost of between $10 and $20 a shot, compared to $100 per Merck dose, the new vaccine would still be too expensive for many Third World countries. A series of three shots, spaced months apart, is usually needed and protection lasts only five years. A better alternative already exists, in the laboratories of the London School of Hygiene and Tropical Medicine, and is poised to go into clinical trial.

Dr. Arie Zuckerman, working with Dr. Kwesi Tsiquaye at the school's Department of Medical Microbiology, used a completely different approach from Merck scientists and Ken Murray's group. Their idea is chemically to "open up" the surface antigens, then reassemble the components into aggregates called micelles. In this looser arrangement all the "antigenic determinants" (the molecular groups within the surface antigen that stimulate antibody production) are exposed.

Not only did this preparation protect chimps challenged with hepatitis virus but much less vaccine was needed, compared to a Merck-type preparation.

Commercial development of the London school's vaccine lies in the hands of the British Technology Group, which owns the patent rights. The development of genetically-engineered alternatives lies with small, struggling companies which need to show a quick profit, so there is a danger of the developing world again missing out.

Merck's subsidiary, Merck Sharp & Dohme International, will transfer to Singapore Biotech the technology to make an anti-hepatitis vaccine. Singapore will immunize its high-risk populations, including newborn babies, hospital workers and military personnel.

Biogen also has developed a potential hepatitis B vaccine in genetically-engineered yeast which it has been striving to develop since 1979.

A genetically-engineered vaccine would also benefit from the nervousness felt about the existing vaccines. All these must be prepared from the blood of carriers of hepatitis B, a chronic disease that causes jaundice and is implicated in liver cancer. Many donors are likely to be homosexuals, the people worst hit by the terrible disease AIDS. (Extracted from The Economist, 23 July 1983, New Scientist, 11 August 1983 and Chemical Week, 14 September 1983.)

Escherichia coli expresses hepatitis B surface antigen directly

Many companies are seeking a recombinant DNA vaccine for hepatitis B, but success has been slow. One of the problems is that the hepatitis B surface antigen is difficult to clone and express in Escherichia coli. Companies that have cloned the antigen generally use other bacteria or yeast. In cases where it has been possible to clone and express the antigen in E. coli the product has not been pure antigen, but a protein formed by fusion of the antigen with another protein. A team at Takeda Chemical Industries (Osaka, Japan) has now been able to clone and express unfused antigen directly in E. coli in considerable quantities. Experimenting with novel plasmids containing the gene for the antigen, the Takeda researchers were able to obtain a low level of expression of the complete surface antigen gene in E. coli. However, the antigen inhibited growth of the bacterium. Removing DNA coding for a hydro-

phobic region at one end of the gene yielded a truncated antigen that did not inhibit growth and was produced in significant amounts. The probable explanation for this is that when the hydrophobic region is present it becomes embedded in the cell membrane and interferes with its activity.

The truncated antigens are not glycosylated nor do they form particle structures as does hepatitis antigen produced in yeast. The antigens are also difficult to separate from the membrane fraction of the E. coli. (Source: Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, N.J. 07024, USA.)

Biogen chooses joint development route for TPA and TNF

Biogen's (Geneva, Switzerland) two new joint development projects point up the difficulties the company will have in implementing its long-term plan to become an integrated company, manufacturing and marketing products from its own research, and even with current assets of over $100 million, it does not yet have the enormous financial resources necessary to commercialize all the recombinant DNA products coming from its laboratories. To this end, Monsanto (St. Louis, MO) will fund Biogen's development of a technique for producing and purifying tissue plasminogen activator (TPA), which is a naturally occurring mammalian polypeptide, which promises to be useful in dissolving blood clots in the treatment of heart attacks, strokes and other circulatory disorders. TPA is available only in minute quantities from mammalian cells at the moment, but recombinant DNA processes could make it available in unlimited quantities. The potential world market may be as large as $1 billion.

Biogen will also develop recombinant microorganisms to produce tumor necrosis factor (TNF) and to scale up production for clinical trials for Suntory (Tokyo, Japan). Suntory will have exclusive manufacturing and marketing rights to TNF in Japan and Taiwan. Development work will take place at Biogen's new Biogent laboratory in Ghent, Belgium.

TNF is another mammalian protein currently available only in small quantities that could be produced in unlimited quantities via a recombinant DNA process. It has been shown to cause destruction of tumor cells in animals and has potential in cancer therapy. (Source: Genetic Technology News, July 1983. Address: 158, Linwood Plaza, P.O. Box 1304, Fort Lee, N.J. 07024, USA.)

Beta interferon clinical trials to begin

Big interest in large-scale production and clinical testing of interferon made by recombinant DNA processes has centered on alpha and, to a lesser extent, gamma interferon, and now clinical trials of recombinant beta (fibroblast) interferon against virus diseases and cancer will begin with a product made by Cetus under an agreement with Shell Oil. Clinical trials of beta interferon from Hoffmann La-Roche (Nutley, NJ) were suspended several months ago because of instability problems. However, Hoffmann La-Roche expects to resume phase I clinical testing of beta interferon shortly.

Cetus researchers attacked the instability of beta interferon by modifying it. The chain of amino acids in beta interferon's molecule contains three units of cysteine. This amino acid contains sulfur, which may form disulfide bonds with other sulfide atoms and contribute to instability. Cetus researchers reasoned that stability would be much improved if one cysteine - the one in the 17 position of the chain - was replaced by another amino acid, serine. The researchers made the modification and got a more stable beta interferon.

Cetus has cloned the gene for modified beta interferon in Escherichia coli. The microorganism is being grown in 1,000 litre fermenters in Cetus's new pilot plant at Emeryville, Ca. If beta-interferon passes clinical tests Cetus and Shell plan to form a joint venture to commercialize it.

Shell Oil is involved in it for two main reasons. First, Shell decided three years ago that it needed to participate in some sort of a recombinant DNA project to assess the technology. Second, Shell thought that a beta interferon venture might open up a new business vehicle for the company (it has no pharmaceutical business at present), but has diversified into veterinary products and agricultural chemicals. (Source: Genetic Technology News, July 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Beta interferon purification requires a specific monoclonal

As interest in beta interferon (β-INF) picks up, interest is mounting in the use of monoclonal antibodies to purify β-INF by affinity chromatography. One such monoclonal has been developed by Daniela Novick and co-workers at Weizmann Institute (Rehobot, Israel). Monoclonals for alpha or gamma interferon won't work with β-INF. The Weizmann team made

hybridomas from spleen cells of mice immunized with interferon from human foreskin fibroblasts. The hybridomas produce immunoglobulins specific for β-INF, which are then bound to agarose-polyacrylhydrazide. Bound material purifies crude β-INF in one step. Serum albumin, often a problem, does not interfere in purification. (Source: Genetic Technology News, July 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Mammalian hybricyte cells produce interferon

Summa Medical believes "hybricytes" have the potential to replace recombinant DNA processes for making interferon and other human proteins. Hybricytes are mammalian cells fused by a method developed at Lovelace Medical Foundation. They were able to produce gamma interferon - or at least a material that shows interferon - like virus inhibition - by the new method. Hybricytes that produce interferon were made by fusing human T lymphocyte cells with myeloma (tumor) cells. The myeloma parent cells pass their ability to grow and reproduce indefinitely on to the fused hybrid cells. The hybricyte receives capability to produce the product wanted - in this case interferon - from the other parent cell. Substituting different types of cells for the lymphocytes should make it possible to produce almost any human protein that might be made via a recombinant microorganism process. For example, pancreas cells could be fused to supply insulin, pituitary cells could yield growth hormone or thyroid gland cells could produce thyroxine. Hybricytes are quite similar to the hybridomas made by fusing spleen cells with myeloma cells to obtain monoclonal antibodies. The big advantage of the hybricyte technique is that cell fusion is hundreds of times more efficient than the usual method applied to making hybridomas for monoclonal antibodies, meaning a big saving in time and money.

The fact that hybricytes produce proteins in their natural, glycosylated form (with carbohydrate side chains) is another plus for hybricytes, since recombinant bacteria cannot do this. So far there is no indication that glycosylated interferons are better than nonglycosylated materials, although glycosylation may be important for other products. Hybricytes might also produce a whole family of proteins rather than a single one. A product with a complete family of related proteins might have some therapeutic advantages. (Extracted from Genetic Technology News, September 1983, address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Cetus begins preclinical testing of interleukin-2

Preclinical animal studies to test interleukin-2 (IL-2) for possible treatment of cancer and acquired immune deficiency syndrome (AIDS) has begun by Cetus who is also supplying highly purified IL-2 to numerous medical researchers around the world for evaluation for a number of other therapeutic applications.

The IL-2 gene was cloned in Escherichia coli and a purification process devised. At the moment, production is in 10-litre fermenters, but the company is scaling up a production process at its pilot plant in anticipation of the need for larger quantities for human trials later. (Extracted from Genetic Technology News, October 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Further doubts cast over interferon's future

Further doubts about the clinical acceptability of interferon have been raised with the remarkable claim that as well as being of limited use in treating cancer, the drug may actually induce the disease. Since the beginning of the year, there has been growing evidence that interferon if failing to live up to its early promise in full clinical trials. Subsequently, several projects have been abandoned because of unacceptable side-effects and disappointing results. And at the Society of General Microbiology meeting in Leeds last month, there was a warning of a worrying, though tenuous, link between the drug and new cancers.

A patient who had been successfully treated with interferon against non-Hodgkin lymphoma (NHL) at the Sloan-Kettering Memorial Hospital, New York recently developed a secondary cancer, myoblastic leukaemia, which was unrelated to the first disease. Although it has not been possible to establish a causal link, a case of this type is highly unusual. The progress of patients in other trials will now need to be followed up in case interferon is found to be mutagenic.

NHL is one of the few types of cancer in which interferon has been used with any degree of success. In one trial of ten subjects, two showed complete remissions of the disease, but this is one of the easier cancers to treat and it is probable that the same success rate could have been achieved with the same degree of discomfort and side-effects using a conventional agent.

The true function of interferon in controlling cancer cell growth may have been misunderstood. It may be more appropriate to use it as a hormone regulating agent rather than as a cytotoxic drug and it may then be possible to treat patients over a longer period with much lower doses which would not induce the same spectrum of side-effects.

The great disappointment caused by the failure of interferon is the result of the unrealistically high expectations of the drug's value generated by early studies. It is only in the past two years that genetic engineering has provided sufficient quantities of interferon to test against cancer, viral infections and the other conditions against which natural interferon had been thought effective. Since then, the normal rules governing the conduct of clinical trials have been broken. Phase II and Phase III trials have been started before the Phase I trials (to establish the maximum tolerable dose and determine toxic effects and pharmacology) have been completed. Much of the blame for this rests with the biotechnology companies which have been involved in an 'unseemly scramble' to recoup their investment costs.

Interferon's performance in trials to test its antiviral effects has been considerably more convincing, but the likelihood of it entering routine therapy seems equally remote. (Extracted from Chemistry and Industry, October 1983.)

Enzymes altered through genetic engineering

A team of British scientists for the the first time used genetic engineering to alter an enzyme. They improved the ability of the naturally occurring enzyme tyrosyl tRNA synthetase to bind with its substrate adenosine triphosphate (ATP), a key step in the manufacture of proteins. Since enzymes are nature's catalysts, controlling key chemical reactions in every living organism, the ability to tailor them at will opens up new possibilities in the manipulation of human biology.

Molecular biologists working at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, used "site-directed mutagenesis" to alter only a small portion of the gene coding for the Tyr TS. Changing the fine structure of a gene represents the latest advance in the genetic manipulation of an organism, which to date has revolved around the transferral of whole genes from one species to another. Dr. Winter's sophisticated genetic engineering technology came into its own only after 10 years of hard work with crystallographer Dr. David Blow, and biochemist Dr. Alan Fersht, at Imperial College, London. Together they built up a three-dimensional model of this enzyme at its amino acid side chains, which gave a clue as to which part of this complicated molecule would have to be changed.

Enzymes act in two stages: first by chemically bonding onto a selected substrate, then catalyzing the reaction of the substrate with some other chemical. Enzymes possess as many as 1,000 amino acids, arranged in a complicated three-dimensional array of active side chains along a backbone.

Changes in the side chains alters the balance of chemical forces that hold the protein's structure in this array, and may alter the properties of the enzyme. But not all of the amino acid side chains are of equal importance to the enzyme's activity, and selecting which one to alter presented Winter, Fersht, and Blow with many headaches.

This then opens up the possibility of redesigning a whole range of proteins, from industrial enzymes to human hormones. For example, glucose isomerase, a common industrial enzyme, much used in the manufacture of liquid sweeteners, has a poor affinity for its substrate glucose.

The American market alone is currently worth millions of dollars a year. There may be other far-reaching applications in health care involving a new generation of vaccines which Winter's team is working on at the moment. (Extracted from New Scientist, 13 October 1983.)

An enzyme that degrades lignin

The full nature of the key step in lignin degradation has been discovered by scientists at the Agriculture Dept.'s Forest Products Laboratory in Madison, Wis. The step - oxidation of carbon-carbon bonds by hydrogen peroxide - is catalyzed by an enzyme and is not, as previously thought, a nonspecific chemical reaction. The work opens the possibility of using genetic methods or recombinant DNA technology to improve the lignin-degrading ability of fungi, and thus the economics of obtaining chemicals from wood and other biomass. The enzyme, isolated from the white rot fungi Phanerochaete chrysosporium, showed good activity in degrading spruce and birch lignin. (Source: Chemical Engineering, 22 August 1983.)

Plasmids code for enzymes that break down nylon waste product

A gene that codes for an enzyme that breaks down a waste product from manufacture of nylon-6 has been cloned by workers at Osaka University (Osaka, Japan). This demonstrated ability of a microorganism to develop new enzymes to react with synthetic substances that had never existed in nature raises hopes for better methods of cleaning up harmful substances in the environment. It also raises hopes for developing entirely new microbial or enzymatic processes for making industrial chemicals.

The gene cloned by the Japanese group is one of two that code for enzymes that catalyze breakdown of 6-aminohexanoic acid dimer (a combination of two molecules of 6-aminohexanoic acid). The genes are contained on a plasmid found in a species of Flavobacterium isolated from wastewater of a chemical plant producing nylon-6. One of the enzymes breaks one of the amide bonds in the cyclic dimer to convert it into a linear dimer - with the two 6-aminohexanoic molecules connected by only one linkage. The second enzyme breaks the remaining linkage to yield two molecules of monomer.

Both enzymes were previously found to be inactive when tested against more than 100 natural linear and cyclic compounds containing amide bonds. This is strong confirmation that the enzymes were developed by the bacterium in response to the presence of synthetic dimer when it was introduced into the environment.

A portion of Flavobacterium plasmid DNA containing the structural gene for the second enzyme was cloned by inserting it into the pBR322 vector for Escherichia coli. (Source: Genetic Technology News, October 1983. Address: 158 Linwood Plaza, P.O. Box 1304, NJ 07024, USA.)

Slow acting enzymes fix nitrogen in modules

Nitrogenase molecules that control the rate of nitrogen fixation by soil bacteria are probably among the slowest-acting enzymes known. According to research at Sussex University, it was found that as the enzyme's activity speeds up, the rate of nitrogen fixation falls. The researchers are studying the kinetics of N_2 fixation by computer modelling, and found that the turnover time of nitrogenase, the crucial enzyme in the process, is one and one-half seconds. Normally, enzymes operate at speeds of mircroseconds or even nanoseconds.

The computer model predicts that quickening the enzyme's rate-limiting action by genetic manipulation, would not give the molybdenum atom time to pick up the extra electron it needs to bind nitrogen - an intermediate step in converting it to ammonia. Instead, it would drive it toward making more hydrogen, and so itself becomes the rate-limiting step.

The molecular biologists at Sussex University are also exploring the transfer of nitrogenase genes from bacteria other than Rhizobium such as Azotobacteria. Their ultimate aim is to transfer this bacterium's nitrogenase genes into plant organelles such as chloroplasts. Rhizobia work only in the confines of root-system nodules in certain plants, notably legumes, where the concentration of oxygen is very low. Azotobacteria possess an extra iron-containing protein that protects nitrogenase from oxygen, and so should be a more suitable genetic-engineering candidate for cereals, such as wheat and barley, which cannot form soil-bacteria nodules. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

An enzyme strips phenols from wastewater

Horseradish peroxidase, an enzyme derived from horseradish when the plant is mixed with hydrogen peroxide, is particularly effective in stripping phenols from wastewater when the mixture is added to solutions containing phenols, solid particles containing the phenols are formed in the liquid and can be filtered out. Current methods of removing phenols, which are major pollutants in wastewater from coal-conversion processes, include solvent extraction and adsorption on activated charcoal. While these methods are reasonably effective, they have shortcomings, such as incomplete purification and formation of hazardous byproducts. (Source: Chemical Week, 20 July 1983.)

Biopolymes through rDNA techniques

Recent developments in microbiology, biochemistry and other biotechnology techniques could result in production of biopolymers of more consistent quality at lower, more stable prices. BioInformation Assoc. and CH Kline are investigating the commercial impact of the

new techniques. Routes used in the synthesis of biopolymers via fermentation can be manipulated to effect changes in fundamental properties, such as chain branching, molecular weight and charge density. Recombinant DNA techniques could be employed to assemble in organisms the enzymes required to synthesize previously unknown biopolymers. Properties of biopolymers, particularly polysaccharides, could be modified by enzymatic or fermentation processing.

Substantial amounts of some bioploymers are used in foods, drugs, cosmetics, paper, textiles and oils. Since many polysaccharide gums imported from overseas are subject to variations in price, quality and climate and dramatic fluctuation in supply, attempts are being made to replace them with synthetics or other natural materials. (Source: Technology Update, 24 September 1983.)

Killer yeasts developed to clear worts

Beer-making involves adding a surprising number of chemicals to the basic mixture or "wort", which worries consumer organizations. British biotechnologists are applying an elegant technique called "rare mating" to develop new strains of yeast to eliminate most of these. The scientists already have strains of "killer yeasts" to clear worts of microbes which spoil the colour and taste, and new types for making low-calorie beer without adding industrial enzymes. Normally, when yeast cells meet and mate, the contents of both the nucleus, which bears most of the cell's genetic material, and the cytoplasm outside, mingle and fuse. So the hybrid possesses a random selection of genetic material from its parents.

Rare mating is much more specific. It involves pairing a conventional yeast strain with a mutant whose nucleus will not fuse. Eventually, through conventional strain selection, a hybrid will emerge, bearing the nucleus from one cell only. It is thus easier to control the genetic properties of the new variety.

Three British groups, one at the Brewing Research Foundation in Surrey, another at the University of Birmingham, and yet another working for Guinness, have announced a new strain of "killer" yeasts which prevent infection by wild yeasts, yet still yield a good brew. The groups constructed these strains by fusing a "nuclear mutant", carrying a gene for a "killer" protein called zymocin in its cytoplasm, with a stock industrial yeast.

The hybrid makes the toxin, which kills off sensitive wild species while retaining most of the properties of a good beer: it is clear, with no sedimentation, has the normal colour and of course flavour. Spoilage either during production or later in the bottle costs the beer industry thousands of pounds in discarded batches or returned bottles.

Making low-calorie beer needs yet another additive, amyloglucosidase. This industrial enzyme breaks down dextrin, a product of starch digestion which yeasts cannot handle, and yields more glucose for the yeasts to digest. The resulting brew has no fermentable carbohydrates, is lower in calories, and better for diabetics who have to watch their intake of carbohydrates. These enzymes come from the fungus Aspergillus niger. In response to pressure from consumers who want a more natural brew, researchers have constructed new hybrid strains, using rare mating, which secrete amyloglucosidase naturally. These enzymes are cheap, but the groups also hope to eliminate the need for papain, another much more expensive enzyme from the exotic paw paw fruit with which brewers now prevent "chill haze".

By incorporating another piece of genetic engineering technology, the economics of some processes in the biotechnology industry that rely on yeasts could be turned around. (Source: New Scientist, 25 August 1983.)

New commercial-scale monoclonal antibody plants

Sensing a growing demand for monoclonal antibodies, such as anti-interferon, Damon Biotech of Needham Heights, MA is spending $3 million on a large new plant. Funds will be taken from the $38 million the company received from its recent public stock offering. Output will also include other monoclonals, for both purification and therapeutic applications. Construction should be finished by the end of the year and the plant will employ 15 to 20 people when it reaches full operating rate.

New plant will use Damon Biotech's patented microencapsulation process. Human or animal hybridoma cells, source of the monoclonal antibodies, will be encapsulated in microspheres and grown in 100-litre fermenters. The microcapsules' semipermeable membranes permit harvesting of a highly concentrated, relatively pure monoclonal antibody suspension, giving the process big advantages over other methods of production. For example, the Damon Biotech process requires 1,000 litres of culture fluid to produce one kilogramme of monoclonal

antibody. To produce the same amount by conventional tissue culture methods would require 100,000 litres of fluid. Or you could grow hybridoma cells in 50,000 mice. In either of the other processes, purification is a costly part of production - perhaps 50 per cent of total cost. With the Damon process, purifcation costs are minimal. Relatively little space is required for the process, only 5,000 ft^2.

Bio-Response's (San Francisco, CA) new plant should be able to turn out a kilo of antibody per month. The new plant, due for completion in December, is designed to use quite a different technique for culturing hybridoma cells and possibly other mammalian cells that produce such things as anticlotting factor, hormones, insulin or interferon.

Lymph from cows will be used as culture medium. The new plant will have stalls for 19 cows, with each cow equipped with a catheter from which lymph is withdrawn. The lymph fluid is piped into an adjoining room where bovine cells and other material are removed and recirculated to the animal. About 80 per cent of the fluid is recycled at this stage. The remaining 20 per cent goes to the culture room, where it circulates through 5 to 10 culture units per cow.

Culture units contain two types of hollow fibre semipermeable membranes. A molecular-scale membrane permits nutrients from the lymph flowing through the unit to diffuse into liquid bathing the growing cells. A more porous, micron-scale membrane permits fluid containing antibodies to pass out of the unit for final processing and storage.

A cell isolation room will permit Bio-Response to apply a proprietary technique to isolate high-producing cells from the culture units cheaply. These can be recycled to the culture units to increase yields.

The two new U.S. monoclonal antibody production facilities will have a companion in Celltech's (Slough, England) new tissue culture facilities for mammalian cells. Present tissue culture fermenter capacity totals 300 litres, but much more capacity will be installed. Individual fermenters are of 100-litre size and there are plans to increase yields of its tissue culture process by conversion from batch to continuous processing. Production scale equipment for continuous processing is expected to be on stream within the next year. (Source: Genetic Technology News, August 1983, address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Commercial firms might make individualized monoclonals against cancer

Cancer research by Ronald Levy at Stanford University Medical Center might open up a commercial opportunity within a couple of years. Although cautious about his results, Levy has had considerable success in using monoclonal antibodies to treat several types of lymphoma and leukemia. The method involves development of a hybridoma that produces monoclonal antibodies used to treat only one patient, but it requires a six months procedure and costs about $50,000 per patient. Levy believes commercial organizations could reduce costs. A commercial operation would be quite different from those that produce large quantities of only a few different antibodies. What is needed is a system to produce a large number of individual hybridomas. Obtaining a hybridoma suitable for treating a single patient requires injecting blood containing the cancer cells into mice, removing their spleens after a few days, fusing spleen cells with myeloma cells to form hybridomas and screening a large number of hybridoma cells to isolate the particular hybridoma needed. Once isolated, the hybridoma can be grown in the body cavity of a mouse to produce the small amount of monoclonal needed for a four-week course. Each step is time-consuming and some are very expensive.

Levy first used this treatment on a patient considered terminal in 1980. Today the patient shows no sign of the disease. So far a total of 26 patients have been treated. Levy has confined his work to relatively rare types of lymphomas and leukemias for scientific reasons. Applying the technique to solid tumors would be more difficult, because less is understood about them. Levy does not foresee that this treatment will replace currently successful cancer therapies. (Source: Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Monoclonal antibodies developed to bind morphine

Monoclonal antibodies have been developed that bind to morphine, but fail to bind peptide endorphins. The antibody also binds to three chemicals in extracts of cow brain, at least one of which competes very successfully with morphine for binding sights on morphine antibodies. The structure of these three chemicals and their function in the brain is not yet known. The brain produces extremely minute amounts of the opioids, hampering research on them. Research at Hebrew University (Jerusalem) also indicates that sweets may cause the

body to release natural opiates. This conclusion is based on the fact that rats who drink excessive amounts of saccharin build up a tolerance to morphine, probably because endogenous opiates block morphine analgesia by occupying receptor sites in the nervous system. The rats drink the human equivalent of 3-4 gallons daily of highly concentrated saccharine solution every day for one month. Studies are now being done to see if other sweets produce these same effect. (Source: Technology Update, 24 September 1983.)

New monoclonal antibody assays

At the 35th Meeting of the American Association for Clinical Chemistry held in New York in July, 600 scientific presentations were made, out of which 16 described new monoclonal antibody clinical assays. Some are listed below:

Luteinizing hormone in urine, presented by Kenneth C. Kasper, Monoclonal Antibodies, Inc., Mountain View, Calif.;

Human growth hormone in serum, presented by Stanley Y. Shimizu, Hybritech, Inc., San Diego, Calif.;

Human heart ferritin in serum, presented by F. Cavanna, Laboratorio di Ricerche Diagnostici Farmitalia Carlo Erba, Milan, Italy;

Disease-related proteins in urine, presented by J.A. Katzmann, Mayo Clinic, Rochester, Minn.;

Theophylline in serum, presented by J. Tim Jones, Beckman Instruments, Inc., Fullerton, Calif.; Helen M. Clements, Ames Division, Miles Laboratories, Inc., Elkhard, Ind.; and John E. Geltosky, E.I. du Pont de Nemous & Co., Inc., Wilmington, Del.;

Thyroid-stimulating hormone in serum, presented by Myung Lee, SmithKline Instruments, Inc., Sunnyvale, Calif.;

Prostatic acid phosphatase in serum, presented by Robin C. Fraser, Monoclonal Antibodies, Inc., Mountain View, Calif.;

Apolipoprotein A-II in plasma, presented by Evan A. Stein, University of Cincinnati Medical Center, Cincinnati, Ohio;

Apolipoprotein B in plasma, presented by Anne Yvonne Maynard, Washington University School of Medicine, St. Louis, Mo.;

Human chorionic gonadotropin in urine, presented by Stanley Y. Shimizu, Hybritech Inc., San Diego, Calif.; and Sarah R. Baxter, Monoclonal Antibodies, Inc., Mountain View, Calif.;

Human chorionic gonadotropin in serum, presented by Robert J. Danisch, Monoclonal Antibodies, Inc., Mountain View, Calif.; H. Garrett Wada, Monoclonal Antibodies, Inc., Mountain View, Calif.; and Stanley Y. Shimizu, Hybritech, Inc., San Diego, Calif. (Extracted from McGraw-Hill's Biotechnology Newswatch, 15 August 1983.)

An immunoassay using mixtures of monoclonal antibodies may be 1,000 times more sensitive than radioimmunoassay in detecting cancer and many other ailments, according to researchers at Columbia and Rutgers Universities. The assay has not been tested clinically, but computer and laboratory studies indicate that combinations of monoclonal antibodies can augment binding sensitivity for proteins. The monoclonal antibodies form circular complexes with the bound protein, giving potential to detect smaller quantities of any protein than can be assayed with RIA. The assay depends on the selection of monoclonal antibodies, since a two fold increase in the separate affinities of two antibodies used to assay for HCG would result in a 32 fold increase in the sensitivity of the co-operative immunoassay (CIA). CIA will allow scientists to develop sensitive assays from individual monoclonal antibodies with low affinity for a protein, but will probably not replace pregnancy tests which detect HCG, nor will it replace RIA completely.

The company of Becton Dickinson has introduced a monoclonal antibody kit for detecting Neisseria meningitidis group B bacteria, bringing the number of meningitis-causing bacteria strains that its kits can be used to detect up to five. Meningitis, is in most cases caused by Hemophilus influenzae group B, N meningitidis groups A, B, and C, and Strepococcus pneumoniae. The test takes only 10 minutes, as against days previously required to grow cultures to identify the organisms. The new kit is Becton Dickinson's first monoclonal antibody product, and is good for 30 tests. It contains antibody coated onto latex beads as well as positive and negative controls. The test is performed on the patient's spinal fluid. The beads clump together if the bacterial antigen is present.

Inveresk Research International (IRI) (Glasgow, Scotland) plans to launch two monoclonal antibody products later this year. First is a diagnostic kit to identify cytomegalovirus, which can be a severe problem if infection arises during pregnancy. The second product is an assay for monitoring urokinase levels in plasma to reduce side effects of thrombosis. R&D on these products was funded by Cogent - a joint venture by two major UK insurance companies, Commercial Union and Legal and General. IRI is 90 per cent owned by the Scottish Development Agency. (Source: Technology Update, August and September 1983 and Genetic Technology News, July 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Coccidiosis vaccine

Contracts to develop a vaccine to prevent coccidiosis, a major parasitic disease which retards growth of broilers and other poultry, have been awarded to three companies by the U.S. Agriculture Department (Immugenetics' subsidiary, Vineland Laboratories, Hoffman-La Roche and Genex). Immunogenetics says that poultry producers spend over $100 million for anti-coccidial preparations, which is more than they spend for any other form of disease control. A successful vaccine, the company says, could cut the cost of anti-coccidial treatment in half. (Extracted from Chemical Week, 20 July 1983.)

Rum works converts its slops into fuel

The world's largest rum distillery has built the world's largest anaerobic bioreactor. The Bacardi Corporation at San Juan, Puerto Rico, have constructed a downflow digester to clean up its waste disposal act, and make methane to power its boilers.

The 3.5 million-gallon fermenter ended its first 12 months of operation in December, 1982 by extracting from 413,800 gallons a day of rum distillery slops over two million cubic feet a day of methane-rich biogas, and removing 79.4 per cent of the wastewater's organic matter. The year's biogas generation yielded the equivalent of 685,550 gallons of fuel oil, and supplied 40 per cent of the entire distillery's fuel need, at a cost of $2.68 per million BTUs. This year, the process is expected to more than double that amount - 1.4 million gallons of fuel oil equivalent - at a cost of $1.50 per million BTUs. Eventually, it is hoped the bacteria-generated fuel gas will meet 90 per cent of the plant's total energy demand.

The installation is a corrugated plastic filter made by the B.F. Goodrich Rubber Co. of Akron, Ohio. This packing medium, upon which a thick film of microorganisms builds up totals 350,000 cubic feet in volume, fills about 75 per cent of the fermenter tank, and serves as a stationary support for the methanogenic bacteria.

The film of immobilized cells, up to one-eighth inch thick, greatly increases the proliferating volume of the active culture, and the chances the bacteria will survive if a toxic substance enters the ferment. Those on the surface might die off, but cells in inner layers are likly to survive. The fixed film also facilitates re-starting the entire operation following a system shutdown.

The process was started in 1980 with material from fresh cow manure. The culture was seeded in a 3,000-gallon pilot plant and exposed to escalating doses of the rum distillery "slops", a dark brown opaque liquid left after the rum alcohol is distilled off from the feedstock of blackstrap molasses and yeasts. It accounts for 65 per cent of Bacardi's effluent, and exerts over 95 per cent of the Biochemical Oxygen Demand (BOD). That measure of dissolved oxygen in waste represents a major factor in water pollution. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

Biotechnology clean-up search

The race toward a lead in biotechnology now depends on perfecting the process of separating and cleaning up products after fermentation. The British government recently took a big step toward filling some notable gaps in such "downstream processing" skills in British industry. The Department of Trade and Industry announced a new "co-operative R&D project" aimed at developing industrial techniques for separating fermenter products. The project, called BIOSEP, involves at least three government departments, 20 researchers and a sum between £1 and £2 million. The government's Warren Spring Laboratory and the Harwell Laboratory, part of Britain's Atomic Energy Authority, will jointly manage the new programme. They will depend heavily on the fermentation and genetic engineering expertise of another government organization, the Centre for Applied Microbiology Research, Porton Down.

Separating small quantities of a very pure product from hundreds of litres of nutrient broth and bacterial cells often accounts for as much as 60 per cent of the total cost of production costs. Traditional methods developed for the dairy industry or for brewing are too crude. The new biotechnology industries depend on a battery of techniques, starting with high speed centrifugation, solvent extraction, flocculation, flotation and sedimentation. The new programme will also tackle refinements in membrane separation and column chromatography. In fact the Harwell team has developed a unique column chromatography system which can handle tens of thousands of litres very quickly. The heart of the novelty is a supportive skeleton consisting of tiny spheres of kieselghur, titanium, cellulases "or anything really" whose porosity can be varied at will by a patented technique. These spheres can be densely packed to support conventional separation gels which trap a product, antibiotics say, without sacrificing the fast throughput of fermenter broth. The system will also support enzymes or living plant or microbial cells. (Source: New Scientist, 7 July 1983.)

Potato virus infections detected through gene probes

British scientists have developed a sensitive test for detecting virus infections in potatoes that could save growers years spent breeding new varieties.

The Plant Breeding Institute at Cambridge have detected three types of potato virus - "X," "Y," and "leaf-roll" with gene probes that are believed will compete well with current radioimmunological methods.

The gene-probe technology was developed at the U.S. Department of Agriculture in Beltsville, Md. against spindle tuber viroids - a potato pest in the U.S. but not in Britain. These viroids are essentially infectious strings of naked RNA.

To screen new potato varieties for resistance to viruses, breeders traditionally infect the year-old plant in their greenhouse plots with virus. then wait another 12 months to see which varieties show symptoms of infection. This tedious time-consuming process has largely been replaced by immunological techniques, using radiolabelled antibodies. These are based on measuring the reaction with an antigen on the coat of the virus particles in sap taken from young tubers.

To prepare the gene probe, the British team started with viral RNA from which they made a DNA copy for insertion into the pBR322 bacterial plasmid. These strands are labelled with isotopic phosphorus, then mixed with the DNA in sap taken from year-old potato plantlets exposed to viruses. The evidence of radioactivity signals viral infection. Such susceptible plants can then be discarded, thus saving growers the capital costs of planting a field of potatoes an extra year. The next step would be to make the test even more economical by labelling the probe with biotin instead of expensive isotopes. Biotin can be detected by avidan, a small protein prepared from egg yolk. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

Dual-purpose marine microbe fixes N_2

A newly discovered bacterium isolated from a wood-boring marine mollusc achieves what genetic engineers have been unable to accomplish - it both digests cellulose and fixes nitrogen. John B. Waterbury, associate scientist of the Woods Hole Oceanographic Institution and his co-workers unveiled their microorganism in the September issue of Science.

The dual-purpose bacteria can utilize a wide variety of compounds as sole carbon and energy sources, including cellulose, carboxymethyl cellulose, cellobiose, glucose, sucrose, succinate, and glutamate. They reduce nitrogen under low-oxygen conditions and require a saline environment for growth. (Extracted from McGraw-Hill's Biotechnology Newswatch, 17 October 1983.)

Use of hydrogen peroxide to digest cellulose

US Department of Agriculture researchers find value in using hydrogen peroxide to digest biomass. Scientists at the Northern Regional Research Center (Peoria, Ill.) have found that peroxide may be a key ingredient in treating waste biomass to produce animal feed, or a starting material for cellulose-to-ethanol processes. Work began as finding the mechanisms of microbial degradation of lignin, and a way to effectively utilize crop residues or wood wastes for a variety of purposes may have been found.

A series of reactions run at the Center use an aqueous mix of 4 per cent biomass solids and 1 per cent of peroxide. The solution's pH is adjusted to 11.5 with sodium hydroxide, and the mass is left to react, with some stirring, for several hours at room temperature. About 50 per cent of the lignin in the biomass is dissolved, along with most of the hemicellulose. After filtration, the cellulose is recovered and dried. Tests at the University of Illinois (Urbana) show very exciting results in feeding ruminant animals; another possible food application may be to convert the cellulose enzymatically to glucose to be used as feed for single-cell protein. Further testing and experimentation are planned at the Center and engineering optimization steps to the process are being examined. (Source: Chemical Engineering, 25 July 1983.)

Embryo sexing in cattle

Dr. Robert Edwards, who with Mr. Patrick Steptoe pioneered in vitro fertilization techniques in the treatment of human infertility, has high hopes that recent advances in molecular biology will soon provide a means of sexing human embryos before implantation in the uterus. This, he says, would be a first step towards an effective programme for the treatment of inherited diseases.

Rabbit embryos have been sexed and reimplanted by excising a piece of trophoblast at a comparatively late stage of embryological development, but the small size of human embryos makes this approach appear difficult if embryos are to be successfully reimplanted. In collaboration with Dr. Jack Cohen, Dr. Carol Fehilly and Dr. Simon Fishel, Dr. Edwards hopes shortly to start using DNA probes to identify human embryo cells containing the Y chromosome; however, this work will not begin until the ethical position in Britain has been clarified. The isolation of single copy DNA sequences specific for the human Y chromosome was reported in Nature last June.

The technique would involve dividing embryos at around the eight-cell stage. One half would be sexed by autoradiography with labelled DNA while the other would be frozen for reimplantation in a subsequent cycle. The portion of the Y-chromosome determining sex in sex-reversed mice has been identified in single cells by Dr. Lalji Singh and Dr. Kenneth Jones, at the University of Edinburgh. Their test, which used satellite DNA from the W chromosome of snakes, is unlikely to be applicable to human cells, but the principle has been shown to be workable.

By looking for sex-specific DNA sequences directly, problems over gene expression in early embryos are avoided. Later it may be possible to develop probes for other gene loci. (Source: Nature, 28 July 1983.)

Bacterial virus will fight frost damage

The recombinant DNA approach to control of frost damage to crop plants, will get some competition from a series of bacterial viruses to be commercialized by University Genetics' new subsidiary, Frost Technology. The company proposes to spray plants with bacterial viruses (phages) that will kill off Pseudomonas syringae and Erwinia herbicola. These two bacterial species normally grow on plant surfaces and contain nuclei, or "seeds", around which ice crystals can form. Without these nuclei crop plants can withstand temperatures several degrees below freezing without damage.

The viruses have been developed by Russell Schnell of University of Colorado (Boulder, CO) and Lloyd Kozloff of University of California at San Francisco (San Francisco, CA). Applying the viruses to plants destroys 90 per cent of the two ice nucleating bacteria within a few hours. Unlike the use of bactericides, detergents or other materials that have been used to kill plant bacteria, the viruses are highly specific for the two species. They cause no ecological damage. So far, work has been confined to greenhouses. Field testing will start this winter. It will probably take another couple of years to bring a commercial product onto the market. The product will consist of virus-infected bacteria grown in fermenters.

The recombinant DNA approach depends upon inactivation of the genes responsible for synthesis of substances that cause ice nucleation within the bacteria. S.E. Lindow, Nickolas J. Panopoulos and Cindy Orser of the University of California (Berkeley, CA) have developed bacterial mutants without ice nucleating genes. They plan to spray plants with these bacteria, which should be able to compete with the natural bacteria and displace them on the plants. National Institutes of Health's Recombinant DNA Advisory Committee (RAC) has given the California group the go-ahead to field test the mutants.

Other genetic engineering companies are also working on the ice nucleation problem. Advanced Genetic Sciences (AGS) (Greenwich, CT) is developing antagonistic bacteria without ice nucleation properties to occupy positions on the plant normally colonized by ice nucleation bacteria. AGS is also investigating enzymes to be used in conjunction with bactericides. AGS supports research at University of California, Berkeley, on the opposite application of ice nucleation molecular biology - use of ice nucleation to enhance artificial snow-making for skiing. Ingene (Santa Monica, CA) has still another approach. The company is also taking a look at a method of interfering with ability of ice nucleation bacteria to adhere to plants. (Source: Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Agrigenetics gets rights to genes that improve nitrogen fixation

Agrigenetics will commercialize a recombinant DNA process, licensed from Oregon State University, that improves the efficiency of nitrogen-fixing bacteria that live symbiotically with soybeans. The recombinant DNA process makes it possible to introduce genes that govern hydrogen uptake (HUP genes) into nitrogen-fixing bacteria that lack them. HUP genes code for an enzyme, hydrogenase, that enables the bacteria to recycle part of the hydrogen produced as a by-product of nitrogen fixation. Production of hydrogen wastes energy - from 30 per cent to 40 per cent of that needed for nitrogen fixation - and saving this energy aids plant growth and seed development.

The recombinant DNA process was developed by researchers at the University which isolated HUP genes from strains of Rhizobium japonicum, the bacterium that fixes nitrogen in soybeans. Large number of the genes were obtained by cloning in Escherichia coli and genes were then introduced into HUP-deficient strains of R. japonicum that were otherwise suitable.

Only about 25 per cent of R. japonicum strains associated with soybeans contain HUP genes. R. meliloti, which fixes nitrogen in alfalfa, and R. trifolii, which fixes nitrogen in clover, produce practically no hydrogenase. Ultimately it is hoped to transfer HUP genes into these species too.

Vector used to transfer HUP genes was not a plasmid but a cosmid, derived from a bacterial virus (phage). Cosmids are better vectors for moving large segments of DNA (48 to 50 kilobase pairs in this case), making it easy to insert the segments into the bacteria to be modified. Once the DNA has been introduced into a bacterium, it may become integrated into the chromosome, the main body of cellular DNA. This integrated DNA is stable, passing from one generation to the next. DNA that does not become integrated may die out after a few generations. (Source: Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Seed company approaches new genetic engineering techniques cautiously

Seed companies form the most likely channel for commercializing higher plants improved via recombinant DNA. A year ago Pioneer Hi-Bred International set up a modest in-house R&D programme to start application of new genetic engineering techniques with crop plants. The company has been doing plant breeding research for 50 years, and its plant breeding programme has been very successful in improving a number of crops, especially corn. Recombinant DNA and other new genetic techniques are primarily considered as a way to help plant breeders in their conventional breeding works by using tissue culture to reduce the 10 to 12 years it now takes to develop a new plant strain by conventional breeding. Tissue culture could permit screening for some traits in single cells or plantlets in the laboratory rather than by growing plants in the field. So far the research group has obtained good results in regenerating whole alfalfa plants from cells or tissues, but any worthwhile monetary return for Pioneer Hi-Bred is more likely to come from improving its mainstay, corn. The company is also attempting to transform corn with recombinant DNA, and is just getting started in a search for vectors or other means of transforming corn cells with single genes. Others have used the Ti plasmid to transform higher plants, however, it does not work with corn, other cereals and grasses.

Once Pioneer Hi-Bred learns how to transform corn with single genes it will try to use the technique to improve disease resistance. The genes might be isolated from a wild Mexican ancestor of corn, or perhaps from plants even further removed. Virus tolerance is probably governed by a single gene, or a small group of genes. Crop yield and stress resistance are probably governed by a larger number of genes. Their action is poorly understood and it will be more difficult to improve these qualities by recombinant DNA. It is not thought worthwhile developing herbicide resistance in corn, since few weed situations limit corn production. Insect resistance genetically engineered into plants might be more worthwhile. Insects such as corn rootworm or the European corn borer are costly to control. Aphid resistance might be easier to develop, since it is found naturally in some corn strains.

Meanwhile scientists warned that chemical companies could distort conventional agricultural practices by breeding genetically engineered seeds resistant to their own weed killers. Ultimately, these companies hope to sell more of their seeds by selling genetically engineered alfalfa or soya, for instance, that is resistant to the herbicide these companies manufacture. This drive for higher profits could have serious consequences.

Testing and planting of the doctored seeds may take a decade before commercially valuable plants will be available, and then the companies could well entice farmers to buy these varities to pay for their investment. It is "natural" for companies to want to make an extra profit on their own chemicals, but in the rush for profits certain more used herbicides which for instance protect wheat and rice, could be ignored by genetic engineers working for industry. (Extracted from New Scientist, September 1983 and Genetic Technology News, September 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Plasmids may be involved in bacterial cellulose formation

Researchers at the Norwegian Institute of Technology (Trondheim, Norway) are studying biosynthesis of cellulose from glucose, and find that, in about 60 per cent of Acetobacter xylinum strains that have lost their natural ability to synthesize cellulose, the bacterium's complex plasmid system has been modified. Wild strains that synthesize cellulose contain a number of plasmids ranging from 16 to 300 kilobases in size. Strains, produced by chemical mutagenesis, with disrupted cellulose production lose some plasmids or gain others. This indicates indirectly that plasmids may play a role in cellulose biosynthesis. (Source: Genetic Technology News, July 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

A biomass power scheme to be tested

Bonneville Power Administration (Portland, Ore.) will spend $700,000 on a two-year demonstration project to study the technical feasibility of generating electricity from biomass residue and waste products in a gasification process being developed by Pyrenco (Prosser, Wash.). Pyrenco will invest an additional $384,000 in the project. The company has been operating a 300-kW-size experimental plant for about 18 months and will expand the capacity to 450kW. In the company's process, biomass is fed to a gasifier and converted to a product that consists mainly of carbon monoxide and hydrogen, with a small amount of methane. The gas is cooled and turbo-charged, passed through a carburetor, and burned in a Caterpillar engine that turns a generator. The process is said to eliminate problems caused by residual tars that have limited the effectiveness of other biomass gasification systems.

Biomass materials to be used include apple pomace, grape pomace, mint hay, and wood chips, and possibly peat and municipal-garbage solid wastes. (Source: Chemical Engineering, 25 July 1983.)

Single-cell protein

Phillips Petroleum has developed new technology to make the single-cell protein (SCP) industry profitable, despite high oil prices. A 1,500 litre fermenter can use various hydro-carbon substrates, including pulp mill sludge, methanol and sugar. All SCP processes depend on finding a microorganism to digest a cheap carbon source, which is then killed. Phillips process uses a yeast which produces 120-150 g/L of solids using methanol or ethanol as a substrate, although sugars could also be used. Envirocon (Vancouver) uses fungi (Chaetomium cellulolyticum) to digest pulp mill sludge, which mill operators usually have to pay to dispose of.

SCP is only marginally economic in N. America, where soybean meal is readily available. Hydrocarbon-rich countries where soy protein is expensive are viewed as prime markets for SCP, although Phillips may also try to capture some of the North American market for specialty proteins such as casein and soya isolates. ICI's Pruteen, which is 72 per cent

protein, is targeted as a substitute for fish meal and milk in animal feed. ICI is currently recovering its operating costs, but is not getting any return on its $119 million investment. Both ICI and Phillips are planning to develop SCP for human consumption. Meanwhile, making SCP economic will be the major goal. (Source: Technology Update, 10 September 1983.)

Waksman Institute extending biotechnology facilities

The Waksman Institute for Microbiology is constructing a new wing dedicated to research in molecular genetics and biotechnology. The combined facilities will accommodate most scientists now working in these areas at Rutgers University and leave room for additional personnel. The new wing will be a three-story 30,000 square-foot structure with a masonry exterior and steel frame located adjacent to the Institute's present fermentation pilot plant. It will almost double the research space and cost approximately $7 million. It is anticipated that the new added laboratories will accommodate a number of independent investigators, however the first new appointment will be that of a distinguished scientist to provide leadership in the area of molecular genetics and to guide the recruitment of additional personnel.

Major competitors for popular rDNA products

Product	Genetic Engineering Organization	Sponsor or R&D/Commercial Partner
Interferon, alpha	Biogen	Schering-Plough
	Genentech	Hoffmann-La Roche
	Collaborative Research	Interferon Sciences/Anheuser-Busch
	John Hopkins University	Toyo Jozo
beta	Cancer Institute, Japanese Found. for Cancer Res.	Kyowa Hakko/Toray
	Cetus	Shell Oil
	Genentech	Hoffmann-La Roche
	Biogen	Schering-Plough
gamma	none	Hoffmann-La Roche
	Biogen	Shionogi (Far East development only)
	Genex	none
	none	Suntory
	Genentech	none
Tissue Plasminogen Activator	Biogen	Monsanto Fujisawa Pharm. (Far East only)
	Chiron	none
	Genentech	none
	Genex (fibrinolytic agent)	Yamanouchi Pharm.
	Integrated Genetics	Toyobo
Blood Factor VIII	Biogen	Kabi Vitrum
	Chiron	Nordisk Insulinlaboratorium
	Genentech	Speywood Labs.
	Genetics Institute	Baxter Travenol
	Integrated Genetics	none

Product	Genetic Engineering Organization	Sponsor or R&D/Commercial Partner
Bovine Growth Hormone	Biogen	none
	Collaborative Research	Akzo Pharma
	Amgen	none
	Genentech	Monsanto
	Molecular Genetics	Amer. Cyanamid
	Bio-Technology General	Eli Lilly
	Genex	none
Hepatitis B Vaccine	Biogen	Green Cross
	Amgen	none
	Chiron	Merck
	Integrated Genetics	Connaught Laboratories

(Source: Genetic Technology News, August 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Bacillus megaterium could become important cloning host

Bacillus megaterium has some promising possibilities as a cloning microorganism for recombinant DNA experiments or production processes. Recently a research team at Northern Illinois University (De Kalb, Il.) have isolated bacteriophage MP 13 for modifying the organism.

Plasmids from B. subtilis and Staphylococcus species have also been introduced into B. megatherium by transforming protoplasts - a technique that has been more commonly used with protoplasts of higher plants.

B. megaterium is distantly related to B. subtilis - the second most popular bacterial cloning host after Escherichia coli. B. megaterium has been used or proposed for industrial production of proteases, amino acids, vitamin B_{12} and riboflavin. It is considered useful to develop genetic information on B. megaterium and other Bacillus species so that a collection of various safe cloning hosts will be available for producing recombinant DNA products. (Source: Genetic Technology News, October 1983. Address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

COUNTRY NEWS

European molecular biology

The question of Britain's continued participation in the European Molecular Biology Laboratory (EMBL) at Heidelberg in West Germany will remain in the balance at least until October. The possibility of British withdrawal stems from a suggestion last November by the Advisory Board for the Research Councils to the Medical Research Council (MRC) to "review" its support of EMBL. Last year that amounted to £0.9 million, slightly less than 1 per cent of the total MRC budget. The British delegation to EMBL spent three days late in July visiting EMBL and its outstation at the Institut Laue Langevin in Geneva and is now preparing its report which is likely to come before the next council meeting in late October. The ultimate decision will have to rest on a mixture of scientific, financial and political decisions. (Extracted from Nature, 25 August 1983.)

Australia

Australian biotechnology programme revised

As proposed by the previous government, the National Biotechnology Scheme would administer A$2.5 million for basic research as well as applied research in projects showing potential for industrial development. Another A$2.5 million from the Australian Industrial Research and Development Incentives Scheme would be made available to industry specifically for biotechnology. The principal change is that the new programme will abolish the dual support system and integrate all biotechnology support. In spite of the indecision about the total amount to be allocated, DST will shortly be advertising the programme and calling for applications. It may wish to gauge the demand and adjust supply accordingly.

The government proposals are heavily biased towards applied projects, at the risk of neglecting research in molecular biology on which new advances are likely to be based. A DST spokesman said that a minimum condition for award is evidence of industry interest, but industrial involvement would be preferable.

In another move aimed at stimulating the country's biotechnology, the government has decided to introduce legislation allowing Australia to accede to the Budapest Treaty, a patent agreement under the UN World Intellectual Property Organization. The need for such access arises because of the technical difficulties of sufficiently describing microorganisms in patent applications. Consequently the present patent law will be changed to enable deposition in an authorized collection to take the place of a full description for patenting. The legislation will lead to the establishment of an international depository in Australia that might also serve as a national collection for the Australian biotechnology industry. (Source: Nature, 23 June 1983.)

Australians regenerate wheat

Dr. William Scowcroft, principal research scientist at the Plant Industries Division of the Commonwealth Scientific and Industrial Research Organization (CSIRO) reported to the Convocation on Genetic Engineering of Plants at the National Academy of Sciences in Washington, D.C., earlier this year, that using somaclonal variation and cell selection, his group continues to improve wheat faster than traditional plant breeding permits and with less difficulty than splicing individual genes.

Aneuploidy (additional sets of chromosomes) is reflected in some of the variants, but is not the primary cause of somaclonal variation. It is thought that in wheat, regeneration of plants from tissue culture causes chromosomal rearrangements, gene conversions, and DNA transpositions.

One of Dr. Scowcroft's new tissue-culture-derived varieties lacks awns, another has a unicomb character and only one or two stalks for better moisture conservation, thus permitting growth in marginal areas. Others form seed heads early or late in the growing season and can be used to take advantage of regional weather differences.

In wheat cell line Y50E P, his group is finding variation in glume (bract) and grain colour, wax, alpha-amylase production, gliadins (storage proteins), and height. CSIRO's new dwarf wheat drastically reduces the proportion of the photosynthate that goes to the vegetative portion of the plant; therefore, there is more for the grain.

The Australian researchers have had preliminary success screening cells and first and second generation somaclonal plants for the following characteristics: heat, disease, and herbicide resistance; 110-day maturity; mineral tolerance, particularly aluminum and manganese; and high/low moisture adaptability. The group is also looking at variations in sugar cane, rice, tobacco, potatoes, corn, and oil-seed rape. (Extracted from McGraw-Hill's Biotechnology Newswatch, 1 August 1983.)

China

Tool enzymes for genetic engineering produced and supplied by the Institue of Biophysics

A biochemical reagent conference was called by the Division of Biology, Chinese Academy of Sciences, in Beijing on 23-26 March 1983 to examine the production of tool (1562 0367) enzymes for genetic engineering and to make arrangements for research and production of tool enzymes in 1983-85. The biochemical plant of the Institute of Biophysics is in charge of the research and production work. It has formally informed all research units of the country that it is ready to supply them with DNA polymerase, polynucleotide kinase, T4-DNA polymerase, polynucleotide phosphatase, DNA ligase, RNA ligase, RNA polymerase, Ribonuclease 1, Ribonuclease TI, DNAase II, λ-DNA, pBR322, EcoRI, BgIII, SaII, alkaline phosphate esterase, and alkaline phosphate diesterase. Moreover, the plant also supplies hexose kinase, creatine kinase, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, malic acid dehydrogenase, lysozyme, protein kinase, etc. The quality of the above items and their availability are guaranteed. (Source: Beijing Shengwuhuaxue Yu Shengwuwuli Jinzhan (Biochemistry and Biophysics No.3, June 1983.)

Biotech teams up with China

Biotech Research Laboratories, a Maryland-based developer and supplier of monoclonal antibodies, has signed what it believes to be the first biotechnology research agreement between a US company and the People's Republic of China.

Under a three-year contract beginning in October the company is to train members of the Shanghai Cancer Institute in the development of monoclonal antibodies at the company's Maryland Laboratories. On their retun to China, the Shanghai staff would develop their own projects, with each partner permitted to use the end products. With China's new policy of encouraging foreign investment, the research collaboration could lead to a decision by Biotech to manufacture its products in China instead of importing them. (Source: Nature, 25 August 1983.)

Federal Republic of Germany

German biotechnology criticized

According to a report from leading scientists, biotechnology in the Federal Republic of Germany needs a shake-up. The report, commissioned by the Federal Ministry of Science and Technology, heavily criticizes the efficiency and achievements of one of the government's two main centres for biotechnology research, and recommends that the two centres be merged. The centres are the Institute of Biotechnology (IBT) in Jülich, near Cologne, and the Biotechnology Research Facility (GBF) in Braunschweig, near Hannover. The committee of investigators found that West Germany was lagging behind many other countries in biotechnology, but the GBF bears the brunt of the criticism.

Several projects at the IBT are praised. For example, one group has succeeded in processing waste liquor from a sugar factory to make ethanol. The first plant is already in production, with an output of 200 tonnes of ethanol a month.

The proposal to amalgamate the two centres at Braunschweig is opposed by the IBT but supported by the GBF.

The committee was particularly damning towards the GBF, saying that most of the research teams there fell below international standards. It suggested that the plant tissue section at the GBF should close. This recommendation was based on expert evidence from biotechnologists from the University of Munich who work in the same field. (Extracted from New Scientist, 13 October 1983.)

University of Munich has electronic DNA synthesizer

The device is about as big as a refrigerator and looks equally insignificant. Since May it has occupied a spot in a basement laboratory of the Institute for Biochemistry at the University of Munich. However, the apparatus is not to be classed with the laboratory glassware: it is the first gene machine of its kind in Europe.

Fully automatic, the device officially named "synthesis automat" spits out highly prized biomolecules of DNA in milligramme quantities spliced as specified by the input programme.

The mechanical production, initially of only short DNA sequences, was possible so early in the history of the molecular-genetic revolution because the scheme for storing genetic information in DNA involves only four letters, and the gene machine is capable of splicing nucleotides into DNA chains per specification faster than any biochemist can do by hand - fast, accurate and overnight.

Strictly speaking, the device is nothing but a synthesis Idiot and is just as practical or impractical as any other laboratory device which takes over a task. Synthesizing DNA is a monotonous assembly-line job. The machine simply saves downtime. The gene machine requires just 18 minutes to add - reproducibly and with good yield - a specified link to the growing biomolecular chain.

Theoretically, the automat working continuously day and night would require about 137 years to reproduce the genetic material of just a single bacterium. A bacterium does twice as much in just 20 minutes. For all the hereditary information of mankind, all of 100,000 years would be required. Such an undertaking could not be accomplished even with a battery of such machines, and there is no reason to do it.

The Institute will use the machine for synthesizing gene fragments for basic research in building small genes for storing information of medically important protein molecules. (Extracted from Die Zeit, 27 May 1983.)

Finland

New company

Commercialization of a secretion vector for cloning rare foreign proteins in Bacillus subtilis is the first project of Genesit Ltd. The vector, derived from the gene for alpha amylase, was developed by University of Helsinki molecular geneticist Ilkka Palva. Genesit is applying for worldwide patents on the invention. (Extracted from McGraw-Hill's Biotechnology Newswatch, 5 September 1983.)

France

New biotechnology building at Institut Pasteur, Paris

In September the industry and research minister Laurent Fabius laid the first stone of a new building to be devoted to biotechnology at the Institut Pasteur in Paris and also launched an "international network for biotechnologies", previewed by the Versailles summit. The network will link France with Japan, Britain and Canada, and the whole European Community through Brussels: its secretariat will be in France. Construction work should be completed during 1986, and although the building will be run by the Pasteur, the institute has promised to co-ordinate its research policies for users of the laboratories with the Centre National de la Recherche Scientifique, INSERM (the medical research council) and INRA (agriculture). Research will straddle the fundamental and the applied, oriented on the one hand to health, and on the other to industry. The international network in biotechnology will establish communications among existing and future research centres, and encourage joint research programmes. An international committee will develop and control such programmes. (Extracted from Nature, 8 September 1983.)

Greece

Biotechnology development programme

After months of delay, Greece finally announced the first step it will take in developing biotechnology knowhow, a key element of the country's push to transform itself into a producer of high technology.

A Biotechnology Development Programme, included in the 1983-1987 Greek Economic Development Plan before Parliament, will center on medical, agricultural, energy and environmental applications. The programme is to be implemented by a Greek biotechnology corporation which will be established this year. (Source: Chemical Engineering, 22 August 1983.)

Hungary

Biotechnology priority plan

The Hungarian government - in particular, the ministries of industry, agriculture and health - is anxious to launch a priority programme in biotechnology. Tentative proposals were made by the government last autumn, just when the scientific establishment was hit by major budget cutbacks suddenly imposed during the summer vacation. It is now informally agreed that the new biotechnology programme should contain not more than five or six projects and that the role of the scientists should be confined to research. For industry, however, which will be concerned with the practical side of the programme, the choice has, to a large extent, already been made. Hungary's industrial research support system allows every enterprise the choice of ploughing back a fixed proportion of its profits into in-house research or else paying the same sum into a central research pool. In practice, most large concerns prefer to do their own research and several pharmaceutical and chemical enterprises are already well launched in biotechnology. (Extracted from Nature, 28 July 1983.)

Increase in biotechnology R&D budget

The Hungarian Council of Ministers added an agricultural and industrial biotechnology research programme to their national medium-term R&D plan. The seven-year plan provides 720 million forints ($17 million) for research and 4 billion to 6 billion forints ($90-$140 million) for application of results.

Hungarian intellectual resources and research achievements are considered adequate for starting projects, but international co-operation - and in certain areas purchase of licenses and know-how - are indispensable. The programme favors joint ventures and free flow of capital, technology, and specialists.

Eight "productive" projects are identified by the programme:

Biogal Pharmaceutical Works, Debrecen
Meriklon Economic Association, Budapest
Gedeon Richter Pharmaceutical Works Ltd., Budapest
Association of the Hungarian Pharmaceutical Industry, Budapest
Phylaxia Serum Works, Budapest
Central Food Research Institute, Budapest
Babolna Farm Combine (West Hungary)

(Extracted from McGraw Hill's Biotechnology Newswatch, 17 October 1983.)

India

Biochemical Engineering activities at the Indian Institute of Technology Delhi began in 1968 in the Department of Chemical Engineering. Initially some facilities to provide a nucleus in this area were created in the form of a post-graduate educational programme with assistance under the Indo-British Collaboration. The present set-up provides the most advanced and modern opportunities for education, training and research in applied and engineering scienes dealing with biological systems. Later, a School of Bioengineering and Biosciences was established in 1974 with two major areas of activities: biochemical engineering and biomedical engineering supported through bilateral collaboration between SFIT-Zurich and IIT Delhi. Various sophisticated and modern facilities for training, research and development studies in biochemical engineering were added. Because of the significance of lignocellulosic biomass as an important energy source, R&D emphasis gradually moved into this area and the development of novel systems became the main concern of research. The unit was then converted into an autonomous centre for Biochemical Engineering and renamed as Biochemical Engineering Research Centre (BERC) in 1976 which continued to operate on a separately approved budget under an independent administrative structure while its academic programmes continued to be associated with the Chemical Engineering Department. In 1978 a small but effective collaborative activity between BERC and a number of French laboratories began and it resulted in some advanced level training of a few post-doctorals at French laboratories. Recently BERC has been given the status of a post-graduate department which enables it to offer an M. Tech. programme in biochemical engineering and biotechnology, besides research activities in various areas of bio-engineering and microbiological sciences,

such as microbial biochemistry, genetic engineering, enzyme and microbial engineering, biological rate processes and bioenergetics. The objectives of the Centre are to provide a model for an integrated approach towards education, research and development work in the field of biotechnology of the nation's renewable resources. It offers courses which are not available elsewhere in India. The Centre runs on a budget approved by a Review Committee represented by various agencies including SFIT-Zurich and GCI, and chaired by the Director of the Institute. Its activities are reviewed every year.

Israel

Cancer risk assessment test

The Israeli company of Orgenics has produced a new cancer risk assessment test which uses genetically engineered bacterium responses to identify potentially carcinogenic substances. The SOS Chromotest, based partly on the Pasteur Institute's research, and developed by a group of scientists affiliated with Ormat, may be used by public health services, monitoring agencies and various industries to screen materials for possible carcinogenic effects. (Source: Technology Update, 17 September 1983.)

Israeli firm to combat mosquitos, and cotton leaf worm

An Israel company Biotechnology Applications Ltd., is hoping for a $1.5 million investment to fund its biological warfare against mosquitos and cotton leaf worm.

The Hebrew university at Jerusalem owns 50 per cent of Biotechnology Applications while Industrial Development Corp. of Haifa holds the other half. The firm's two insecticidal projects involve scaling up and genetically engineering the microorganism, Bacillus thuringiensis var. israelensis (BTI), which was discovered in this country some five years ago. It secrets an endotoxin described as having unmatched effectiveness against mosquitos.

The company is nearing completion of a feasibility study for industrial-scale production of BTI in Israel, and expects to produce several hundred tons of the bacterial toxin, with antimalaria programmes.

In order to fight cotton leaf worm, the company is working with a variant of BTI toxin that attacks the insect's midgut. (Extracted from McGraw-Hill's Biotechnology Newswatch, 17 September 1983.)

Italy

Italian company funds British research

An Italian pharmaceutical firm has backed with £40,000 ($61,000) a British research team aiming to clone - and develop a diagnostic kit for - a gene thought to play a role in heart disease. Farmitalia Carlo Erba of Milan is supporting work at the Sir William Dunn School of Pathology at Oxford on apolipoprotein A-I (apo A-I), the major protein of high-density lipoproteins which are thought to act as shuttles that transport cholesterol from peripheral tissues to the liver, where the molecule is broken down and excreted. Cholesterol is thought to contribute to causing atherosclerosis. A major deficiency or absence of apo A-I has been associated with very low HDL levels and severe atherosclerosis.

The British team, led by Dr. Tito Baralle, earlier made a cDNA probe to the 243-amino-acid apo A-I. They are now attempting to clone the apo A-I gene in Escherichia coli, and eventually hope to develop a diagnostic kit.

The project is part of a larger programme to investigate the potential of genetic screening for heart disease. At St. Mary's Hospital, London, Dr. Stephen Humphries is looking at the relationship of two other apoproteins to genetically inherited forms of heart disease, particularly familial hypercholesterolemia, in which a person's serum cholesterol is typically two-and-a-half times normal. (Extracted from McGraw-Hill's Biotechnology Newswatch, 15 August 1983.)

Japan

Human stress hormone cloned in E. coli

A major brain hormone in the human stress response, which was identified in animals just two years ago, has now been expressed in bacteria. Professor Seisaku Numa and co-workers in the department of medicine and chemistry at Kyoto University here used a sheep cDNA probe for corticotropin releasing factor (CRF) to extract its counterpart from a human gene library inserted in E. coli.

CRF, a hypothalamus hormone, orchestrates key events in the stress response, including mood and awareness, heart rate, blood pressure and adrenalin and steroid secretion, and is implicated in stroke, heart, and kidney diseases. Therefore an abundant source of this elusive molecule is the first step in developing a means to counteract its effects.

Prof. Numa's recombinant-DNA human hormone is 41 amino acids long, but its sequence has different amino acids at seven sites compared with the sheep molecule. The cloned product also includes a 24-amino-acid tail at the N-terminal position, which is a signal for secretion. There is also an 800-base-pair-intron, but it occurs in a nontranslational area and does not affect the structural gene. In order to clone the CRF gene, the team made a cDNA copy of messenger RNA extracted from sheep hypothalamus glands and inserted the reverse-transcriptase-made DNA fragments into E. coli via plasmids. After screening more than a million of the resulting hosts with a probe based on a partial sequence of the amino-acid structure for CRF isolated by Dr. Vale of the Salk Institute in 1981, the Japanese scientist found one E. coli colony that contained the appropriate sheep cDNA. This he used as a probe to find the human CRF gene in the gene library at Harvard University, Cambridge. One of the cultures contained a part of three human CRF genes. These were spliced and cloned in E. coli using phage vectors. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

Biogas generator runs on rice husks

A biogas generator that runs a small engine by burning rice husks and other waste has been developed by a researcher at Miye University. The Type TK generator partially burns husks and stalks to produce carbon monoxide, distills and filters the gas and then burns it to power the engine. A 7 hp gasoline engine was kept going for one hour at a maximum 4.44 hp by a 280 lb. load of rice husks. (Source: Technology Update, 10 September 1983.)

Breakthrough in protein manufacture

Japanese researchers have overcome one of the main obstacles holding up the mass production of genetically engineered proteins. Their technique is to use Escherichia coli, both to make large quantities of protein and to help in its purification which could allow industrialists to manufacture pure proteins, such as insulin, in sufficient quantities to make it economically worthwhile, thus opening up large new markets for Japanese industrialists.

One of the problems of using E. coli to create proteins is that its outer membrane stops much of the manufactured protein from leaving the bacterium and passing to the surrounding medium, where it can be collected and purified.

The researchers have succeeded in identifying and using a gene that helps the desired protein cross the outer membrane of E. coli. Without the gene, the membrane prevents up to 80 per cent of the protein from getting out of the cell. With the gene, 90 per cent of the product is secreted into the culture fluid. As yet, the team does not understand how the gene acts, but it is certain that the membrane is not broken.

The discovery came from a team led by Dr. Koki Horikoshi, the chief researcher at the Japanese government's Institute of Physical and Chemical Research in Wakoshi, near Tokyo. The team spliced the gene for penicillinase into the DNA of E. coli in the normal manner, using restriction endonuclease that has the ability to cut DNA at specific points along its chain. What is new is that the researchers have spliced a second gene into the E. coli DNA. It was found that, with this gene, the resultant hybrid plasmid (the DNA of the bacterium) was dramatically effective at increasing the yield of penicillinase - 90 per cent of the penicillinase produced was secreted into the surrounding medium. The product was not only pure, it was also easier to collect.

Though still some way from being used on a commercial basis, this new Japanese technique has immense potential. If it can help produce a protein such as penicillinase, then it may also be used to help produce other useful proteins such as insulin, interferon or growth hormone. (Extracted from New Scientist, 29 September 1983.)

British biotechnology seeks Japanese outlet

Celltech, Britain's only gene-splicing company, has signed an agreement with Sankyo, Japan's second largest pharmaceutical company, to cover the commercial exploitation of three drugs. One controls blood clotting, the other two are for healing broken bones in old people. The market for all three is worth more than £100 million a year. This is the first of several deals to be announced in the near future as Celltech begins to reap its commercial rewards from its world position in biotechnology.

The British government and a consortium of private investors set up Celltech in 1980. It already earns money from a system for purifying interferon and blood typing kits. But these modest revenues pale in comparison to what could be earned from the new products: tissue plasminogen activator (t-PA), calcitonin and katacalcin.

Markets for both products already exist in Japan, but potential sales could be much higher if they become available cheaply, through genetic engineering. Celltech has already spliced or "cloned" the human genes into bacteria, but much work remains to purify the product. The company will also have to improve levels of production and design an efficient process on a larger scale.

Under the agreement, Sankyo will pay Celltech the full cost of development work on two products for two years. In exchange for the rights to the worldwide licence, it will pay an extra fee and royalties on sales. (Extracted from New Scientist, 15 September 1983.)

Microbial sweetener

Tokyo - A microbial alternative to an expensive chemical process has been found by Hisao Takemoto and co-workers at the Toyo Soda Manufacturing Co., Ltd. Strains of bacteria to make aspartame (a low-calorie sweetener rapidly replacing saccharine in diet soda) have been isolated. The microbes enzymatically convert an amino acid and a related compound, both costing a few dollars a pound, into aspartame which sells for some $100 a pound.

Aspartame received U.S. Food and Drug Administration approval this summer and has been approved in Japan, is 180 times sweeter than sugar and has none of the bitter aftertaste of saccharine.

The group identified at least two species of aspartame-synthesizing bacteria, Pseudomonas putida and Alcaligenes faecalis, plus the fungus Sporobolomyces odorus. Working with P. putida TS-15001, they found that five grammes of resuspended bacteria produced 0.1 gramme of aspartame after six hours at 37°C. The culture medium initially contained 3.3 grammes of aspartic acid and 4.5 grammes of phenyl-alanyl methyl ester, the unconnected components of the dipeptide sweetener. As a bonus, this bacterial stock can make aspartic acid from one of its carbon sources, fumaric acid. At present the microbial output is low. However, by precipitating out the product, as is done in the chemical synthesis, it may be possible to shift the reaction equilibrium to increase yields. (Extracted from McGraw-Hill's Biotechnology Newswatch, 5 September 1983.)

Team develops microbiological cell

A microbiological electric cell, claimed to be at least twice as effective in electric power generation as the best conventional equivalent, is to be developed by a joint research team from the Tokyo Institute of Technology and Mitsubishi. The cell is based on the idea that sugar molasses can be made to produce a large amount of hydrogen to generate electricity if processed by bacteria and assisting photosynthetic type of bacteria. The research team plans an initial cell of 1W capacity, building up to a 10-W cell suitable for low-voltage power sources. (Source: World Solar Markets, July 1983.)

Japanese boost germs' defenses to screen antibiotics

By increasing the copy number of the genes or using strong promoters, Japanese researchers have boosted the enzyme defenses of some cells as much as seventy-fold. For example, the enzyme cephalosporinase attacks the beta lactam ring of the antibiotic cephalosporin, thereby inactivating the drug.

The Japanese researchers are looking for antibiotics that can withstand this increased biochemical onslaught, yet still kill bacteria. Their cloning results to date are tabulated below.

TABLE

GENE CLONED & SOURCE	HOST(S), VECTOR, SIZE OF CLONED DNA	ADDITIONAL DATA	REPORTED BY
Cephalosporinase, *Citrobacter freundii*	*C. freundii* and *E. coli*, pMK1, 1.5 Kb	In *C. freundii* at 20°C fifty- to sixty-fold higher expression using penicillin as inducer, some expression at 30°C without inducer; in *E. coli* expression 4% of that in wild-type *C. freundii*.	Tetsuo Sawai, Chiba University, Chiba
Cephalosporinase, *Escherichia coli*	*E. coli*, pMB9, 6.0 Kb	Thirty- to seventy-fold higher expression than wild-type; cephalosporinase promoter yielded higher transcription efficiency than tetracycline promoter.	Hitoshi Takanomi, Ichiro Aramori, Fugisawa Pharmaceutical Co., Ltd., Osaka; Sachiko Goshima, Toho University School of Medicine, Tokyo
Penicillinase, *Klebsiella pneumoniae*	*E. coli*, pACY184, 2.3 Kb	—	Matsuhisa Inoue, Gunma University Faculty of Medicine, Gunma; Susumu Mitsubashi, Episomic Research Institute, Gunma

(Extracted from McGraw-Hill's Biotechnology Newswatch, 5 September 1983.)

Animal mutants for use in research

Japan will develop genetically tailored animal mutants for use in cancer and other disease research. Ordinary healthy animals are often unsuitable for such purposes. The Science & Technology Agency will place orders with the Central Institute for Experimental Animals and the Mitsubishi-Kasei Institute of Life Sciences for practical attempts to produce such animals. One method of producing mutants involves removing fertilized ova from two different breeds of female animals, growing each in a test tube until it divides into 8 cells and then combining them. Another technique is to inject the geminal disc taken from a pregnant mouse with a kind of cancer cell known as tetracarcinoma and replacing it in the original womb. Other disease cells could be injected to produce other types of mutants. (Source: Technology Update, 16 July 1983.)

Japanese/American co-operation

Co-operation between the Institute of Physical & Chemical Research and Washington University on monoclonal antibodies and human hybridomas will begin shortly. Co-operation is part of the international scientific technology research co-operation agreed on in 1982 at the Versailles summit meeting. Research will focus on manufacturing and refining technology for monoclonal antibodies, manufacturing and storage technology for human hybridomas and applications for monoclonal antibodies. The Institute for Molecular & Cellular Biology, Osaka University, the Institute of Medical Science, Tokyo University and the University of Chiba will co-operate in the project. (Source: Technology Update, 30 July 1983.)

Government regulations regarding microbial cultivation

The Japanese government has eased regulations on recombinant DNA experiments involving microbial cultivation in tanks of 20 litre capacity or more. Case-by-case authorization will no longer be required, however large-scale laboratories handling microorganisms and genes known to be safe (LS-1) must provide adequate measures to prevent microbial escape into the environment and safety must be checked daily. Research covered includes mass production of insulin, interferon, vaccines against influenza and hepatitis, and other medically useful proteins. Laboratories handling potentially dangerous pathogens (LS-2), like leukemia and encephalitis vaccines, must continuously monitor safety arrangements and a detailed record of each experiment must be available for government inspection for a five-year period. Large-scale work in unspecified categories must still receive case-by-case authorizations. (Source: Technology Update, 3 September 1983.)

Monoclonal to detect and purify TNF

To detect and purify an elusive antitumor drug, Japanese researchers have for the first time developed a monoclonal antibody for rabbit tumor necrosis factor (TNF), which is a mammalian protein that destroys some types of cultured cancers, such as malignant human lung and melanoma cells, in vitro but has no effect on normal cells. Unlike interferon, it has no species specificity, does not involve the immune system, and directly attacks cancer cells. Hideo Naruuchi of Tokyo University's Institute of Biological and Pharmaceutical Testing and Development, and Katsumara Haraneka of the pathology and pharmacology department, both at the Medical School, who developed the antibody, plan to "mass produce" TNF by gene splicing. They will use the monoclonal antibody first to screen for expression of the protein in the recombinant hosts, then to purify the factor by linking the antibodies to a chromatographic column. Currently only limited amounts of animal TNF are available.

To produce their monoclonals, the group first activated the rabbits' immune systems by infecting them with anaerobic microorganisms, Corynebacterium sp., and then challenged the animals with a bacterial toxin that induced them to produce TNF. Mice were injected with the rabbit TNF, and their antibody-producing spleen cells were fused with malignant mouse bone-marrow cells to produce the monoclonal-antibody-generating hybridoma cell line. (Extracted from McGraw-Hill's Biotechnology Newswatch, 1 August 1983.)

Some Japanese R&D biotechnology firms

Company	Main products	Work underway	Partners
Ajinomoto Co.	Foods, amino acids	Cloning of interleukin-2	Cancer Inst. of Tokyo
Asahi Chemical Industry	Various chemicals	Gamma-interferon, tumor-necrosis factor	Dainippon Pharmaceutical Co.
Dainippon Ink and Chemicals	Specialty chemicals	Foods by cell fusion	
Denki Kagaku Kogyo K.K.	Cement, chemicals	Microphage-migration inhibition factor (for cancer applications)	
Green Cross Corp.	Drugs	Alpha-, beta-, gamma-interferon, leucocyte-stimulating factor	Several
Hoechst Japan Ltd.	Drugs, specialty chemicals	Monoclonal antibodies	
Japan Synthetic Rubber Co.	Rubber	Cancer-diagnosis agents Cell cultures	Institute of Immunology Hana Biologics Inc.
Kanebo Ltd.	Fibers and textiles	Drugs by cell fusion, cell culture and fixed enzymes	
Kuraray Co.	Fibers	Drugs by genetic engineering	
Kanegafuchi Chemical Industry	Plastics, specialties	Insulin, interferon	
Kao Corp.	Detergents, toiletries	Foods by genetic engineering	
Kikkoman Corp.	Soy sauce	Soy sauce via bioreactor processes	
Kirin Brewery Co.	Beer	Yeasts by cell fusion and genetic engineering	
Kyowa Hakko Kogyo Co.	Amino acids, drugs, beverages	Beta-interferon tissue plasminogen activator	Cancer Inst. of Tokyo
Lion Corp.	Detergents, toiletries	Biomass by fixed enzymes, plant-growth hormones by genetic engineering	
Meiji Seika Kaisha Ltd.	Antibiotics, confections	Interferon	G.D. Searle and Co.
Mitsubishi Chemical Industries	Various chemicals	Endorphin via genetic engineering, monoclonal antibodies,	
Mitsubishi Yuka Pharmaceutical Co.	Drugs	Urokinase by cell culture	Nippon Abbott K.K.
Mitsui Petrochemical Industries	Petrochemicals	Drug ingredients via cell culture	
Mitsui Toatsu Chemicals, Inc.	Various chemicals	L-tryptophan via enzymatic reaction	
Nippon Paint Co.	Paints, coatings	Drugs, via cell culture	
Nippon Roche K.K.	Drugs, toiletries	Interferon	
Nippon Zeon Co.	Rubber, polyvinyl chloride	Synthetic DNA	
Oji Paper Co.	Pulp and paper	Tree-growth factor via protoplast separation, cell fusion	
Otsuka Pharmaceutical Co.	Drugs	Interferon, via cell fusion	
Sagami Chemical Research Center	Drugs	Peptides for cancer research	
Sankyo Co.	Drugs	Drugs by cell fusion, genetic engineering	
Sanyo Chemical Industries	Specialty chemicals	Clinical reagents, via fixed enzymes	
Sapporo Breweries Ltd.	Beer	Yeasts by cell fusion	
Shinogi and Co.	Drugs	Insulin	Eli Lilly & Co.
Showa Denko K.K.	Various chemicals	L-tryptophan, via fermentation	
Sumitomo Chemical Co.	Various chemicals	Interferon human growth hormone growth-hormone releaser	Wellcome Foundation KabiVitrum Salk Institute
Suntory Ltd.	Beverages	Drugs, including interferon	
Takeda Chemical Industries	Drugs	Drugs, including interferon	F. Hoffmann-LaRoche
Teijin Ltd.	Fibers	Monoclonal antibodies	Hybritech Inc.
Toray Industries Inc.	Fibers	Drugs, including interferon	Daiichi Seiyaku Co., Genentech Inc.
Unitika Ltd.	Fibers, textiles	Adenosinetriphosphate, monoclonal antibodies	Institute of Physical and Chemical Research
Yamanouchi Pharmaceutical Co.	Drugs	Alpha-interferon	Essex Nippon Co.

(Source: Chemical Engineering, 22 August 1983.)

Mexico

Biotechnology centre to open next year

Construction of the Research Center for Genetic Engineering and Biotechnology at Cuernavaca will be completed this year. Research staff are expected to start moving in early 1984. The centre, emphasizing applied research aimed at specific Mexican needs, will work on 16 projects including nitrogen fixation, amino acid and single-cell-protein production, microbially enhanced oil recovery, and microbial electrodes, and possibly vaccine biosynthesis. (Extracted from McGraw-Hill's Biotechnology Newswatch, 1 August 1983.)

Netherlands

University to receive 2.5 million guilders annually for biotechnology research

The Netherlands government announced it will grant the University of Amsterdam 2.5 million guilders ($845,000) annually over the next decade for biotechnology research, expected to focus on pharmaceuticals and fermentation. The funds are part of a 70-million-guilder sum earmarked by the government for biotechnology through 1988. Approximately half the money will be spent on applied research. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

Dutch researcher uses bacteria to extract sulfur from coal

In a bench-scale reactor, microorganisms removed 90 per cent of the sulfur from a sample of coal in two weeks.

T.F. Huber of Delft University built a system made up of a 10-litre mixed-flow reactor, in which a bacterial culture dominated by Thiobacillus ferrooxidans proliferated, connected to a plug-flow reactor where the culture continued to oxidize most of the sulfur in the coal to soluble sulfates. He found, after conducting five experiments with 16 per cent to 19 per cent w/v coal slurries containing 2.3 per cent sulfur, that in the mixed-flow reactor a correlation existed between bacterial growth and pyrite (RdS_2) oxidation, the quantity of organisms determining to a point the oxidation rate. Their growth rate being 0.053/h, the bacteria worked most efficiently with a residence time of at least 20 hours. In the plug-flow reactor, an increasing divergence between bacterial growth and pyrite oxidation was found - growth stopped after about 130 hours, but oxidation continued - deducing pyrite-surface-limitation as the cause. The Dutch Project Office for Energy Research supported work as part of a programme aimed at finding a way to take sulfur out of the hydrocarbon before burning it as fuel. Such removal could eliminate some of the drawbacks of using the coal as an energy source, such as the compound's possible link to acid rain. (Extracted from McGraw-Hill's Biotechnology Newswatch, 1 August 1983.)

United Kingdom

British government's second biotechnology company

Agricultural Genetics Corporation (AFC), the British government's second venture into the biotechnology business, is backed by the British Technology Group, the oil company Ultramar, the investment group Advent Technology and Advent Eurofund. It will have a first option to exploit work carried out at five of the Agricultural Research Council's research institutes.

The company will work in three areas: nonconventional plant breeding (using genetic engineering for instance), microbial inoculants to replace fertilizers, and biological pesticides and herbicides. (Extracted from New Scientist, 28 July 1983.)

Blood proteins research receives little government support

Blood proteins is an area of genetic engineering research in which work in Britain matches the best in the world, is not getting the government funding it needs, and depends heavily on voluntary donations which are expensive and unsafe. According to a spokesman of Speywood laboratories, genetically engineered products could be a viable alternative, making paid donors a thing of the past, yet in Britain there is little sign that they are investing in the essential R&D necessary to enter this market.

It is estimated that the current world market for blood products from the traditional source, human plasma, is worth about $1 billion a year. Products include human serum

albumin, used as a blood extender for treating severe burns, and blood factors VIII and IX. Britain spends millions of pounds each year importing freeze-dried plasma from the United States, which these days carries a small risk of conveying auto-immune deficiency syndrome, as well as hepatitis, which genetically engineered proteins should eliminate by providing pure protein with fewer side effects.

The American genetic engineering company, Genentech, was the first to splice the gene for human albumin, first into bacteria, then into yeasts. But, this nation of beer drinkers has produced some of the finest experts in the world in continuous fermentation (capable of running for months at a time) which seems the logical approach to producing the hundreds of tonnes of albumin that will be required. (Source: New Scientist, 25 August 1983.)

Celltech links with Sankyo

Celltech has granted licences to the Sankyo Company of Tokyo which give it world-wide marketing rights for two therapeutic products that will be developed at Celltech's laboratories in Britain. Celltech will receive licensing fees, development costs and royalties.

The two products are human tissue plasminogen activator and calcitonin, a hormone involved in calcium regulation. Celltech has already cloned genes for both molecules in bacteria and hopes to have the products on the market within four years. Sankyo will be responsible for scaling up production on commercial levels. The two companies will also collaborate in research on katacalcin, a recently-discovered hormone with properties similar to calcitonin.

Tissue plasminogen activator (t-PA) is a thrombolytic agent that has potential to replace urokinase and streptokinase, both of which have the disadvantage that their action is relatively unspecific and that they may stimulate a troublesome immune reaction. The Japanese market for thrombolytics is valued at £100 million a year, although such products are much less widely used in the United States. But a cost effective replacement with the advantages of t-PA might change that. At least two other companies, Biogen in Switzerland (collaborating with Fujisawa in Japan) and Genentech Inc. in the United States are also working on human t-PA.

Calcitonin is at present used in treating Paget's disease and hypercalcaemia. It may also prove to be useful for osteoporosis, which is a disease of considerable economic importance, being the cause of many bone fractures in older women. (Source: Nature, 15 September 1983.)

Britain stays cool over genes in court

British authorities seem unconcerned about the prospect of a court battle over gene-splicing. No work similar to that under way in the US is contemplated by the government-funded Argicultural Research Council (ARC), and industrial projects, if there are any, have not reached the field-trial stage, according to a spokesman of the Genetic Manipulation Advisory Group (GMAG) which advises the government on recombinant-DNA matters. The GMAG and other government groups decided last year that the question of releasing genetically manipulated microbes to the atmosphere or planting genetically-tailored plants outside would be decided on a case-by-case basis.

Dr. Dick Flavell, a plant biologist at the Plant Breeding Institute in Cambridge, thinks that in any case the question of potential damage to the environment revolves around how robust these microbes will be in competition with the thousands of bacteria that normally infect the soil, lie on plants and float in the air.

Ordinarily these strains of psuedomonas invade the plant cells at a certain temperature, then secrete some chemical which precipitates the formation of ice cyrstals around them. These ice cyrstals physically rupture the cell membranes, causing frost damage. The basic principle of the American experiment is to delete the gene in the bacteria which programmes the production of this chemical so the neutered bacteria will no longer "seed" ice crystals. (Source: New Scientist, 22 September 1983.)

The British picture

The opening up of Ministry of Defence research establishments and the removal of the British Technology Group (BTG) monopoly over government-funded research is unlikely to result in a flood of new products.

One of the most important differences between the United Kingdom and the US in the development of high-technology companies, is that British companies cannot offer the same share option schemes as their competitors across the Atlantic. Increases in share values under these schemes in Britain are treated as income, incurring higher tax rates, especially for highly paid key employees, than in America, where they count as capital gains, and accordingly taxed at a lower rate. This is even more disastrous for the fledgling biotechnology companies, of which there are very few in Britain compared to the US. But change is on the way. The first target will be Celltech, Britain's only genetic engineering company supported largely by government money and a consortium of private investors. At the moment no more than a few per cent of the total share capital is in staff hands, but this may shortly be "slightly extended". However, this nowhere matches companies such as the San Francisco-based firm Cetus with reportedly 12 per cent of its share capital in staff hands.

A source within the British Government's programme on biotechnology admits that the change in BTG's role will create more freedom and "a wider set of opportunities" but he thinks that safeguards are necessary to make sure that publicly funded research does not end up being exploited by foreign companies. On the other hand, Ronald Colman (director of the Laboratory of the Government Chemist and head of the DoI's Biotechnology Programme) said that biotechnology is part of an international market for skills and ideas, and that one cannot expect safeguards. What one should do is create a climate in which people will want to work. (Extracted from New Scientist, 22 September 1983.)

U.S.A.

Navy launches five-year R&D programme to make strategic biomolecules

The U.S. Navy began a five-year, $13-million programme using biotechnology. To explore what biotechnological areas the Navy should investigate, the National Research Council (NRC) brought together 27 academic and two industrial biotechnologists at a Conference on Biomaterials.

The Navy's needs include immobilized enzymes and whole cells, molecules used in adhesives, coatings, lubricants, sound and chemical sensors, and industrial-scale chemical processes. The Navy will do its own in-house biotechnological research at the Naval Research Laboratory in Washington, D.C., the Naval Bioscience Laboratory in Oakland, Calif., and the Naval Air Development Center in Warminster, Pa., contracting to industry and universities research with scientific merit and relevance. If any of the contract work is patented, the patent will be assigned to the inventor, with the Navy retaining rights to use the process or product royalty-free.

In fiscal year 1985 the naval Air Systems Command - which is funding the five-year R&D programme along with the Office of Naval Research - will examine the research findings.

Research will cover a whole host of subjects ranging from medicine, corrosion control, food production, lubricants, sound and chemical sensors, enzymes, adhesives and waste disposal. (Extracted from McGraw-Hill's Biotechnology Newswatch, 3 October 1983.)

The American Type Culture Collection

The American Type Culture Collection, a non-profit making organization established in 1925, houses one of the most diverse collections of strains in the world with over 31,000 different strains of algae, bacteria, bacteriophages, fungi, plant and animal viruses, antisera, protozoa and cell lines, plus a rapidly growing collection of plasmids, recombinant DNA vectors and hybridomas. Areas of research focus on comparative microbiology, microbial systematics, computer-assisted identification, freeze-drying of microorganisms and biological reagents, culture safekeeping, plasmid preparation, mycolplasma testing, karyotyping and virus purification. In addition the ATCC serves as a patent culture repository as well as a cell information bank and hybridoma bank. The ATCC has also recently begun a workshop series encompassing a variety of topics. Address: 12301 Parklawn Drive, Rockville, Marylar' 20852, USA.

Center for Crop Molecular Genetics

Center for Crop Molecular Genetics and Genetic Engineering will be set up at University of Illinois (Champaign, Il) with funds from SOHIO - Standard Oil Co. (Ohio) (Cleveland, Oh). The centre will receive about $2 million over five years. Programmes will focus on molecular genetics of major crops, particularly corn and soybeans. (Source: Genetic Technology News, September 1983, address: 158 Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

Genentech links with Hewlett-Packard

The Californian biotechnology company Genentech has signed up with Hewlett-Packard to develop a range of instruments for use in the industrial application of biotechnology. The new organization, whose creation was agreed on 20 July, will be managed by Hewlett-Packard, which will be the major owner of the new company and which expects to contribute $10 to $20 million in the next five or six years.

While gene synthesizers and similar equipment may provide the new company with something to make in the immediate future, both partners emphasized that they have set their sights on the development of instruments that will have a natural place in scaled-up production processes. (Source: Nature, 4 August 1983.)

Genex links with Yoshitomi

Yoshitomi Pharmaceutical Industries (Japan) will jointly develop genetically engineered human interleukin-2 with Genex (US). The immune modulator may be useful to treat acquired immune deficiency syndrome (AIDS). Immune modulators control growth, differentiation or functioning of cells in the body's immune system. Human interleukin-2 is produced by white blood cells, and may promote the growth of thymus-dependent lymphocytes or T-cells within and outside the body, thereby acting as a virus-fighting agent. The substance may also prove useful in laboratory work on cancer therapy and in immunodiagnosis. (Source: Technology Update, 3 September 1983.)

USSR

Biotechnology effort

While many Western companies have entered the field of biotechnology to produce engineered genetic materials, the Soviet effort has been far more basic. The main objective of the U.S.S.R.'s Chief Administration for the Microbiological Industry (referred to as Glavmicrobioprom) is the mass production of feed proteins, amino acids, and diet supplements for the country's livestock industry, one of the most undernourished in the world.

A study recently put out by the OECD notes that if the USSR could raise the amount of digestible protein in an average kg. of animal feed from the present 85 g to 110 g, the country's feed-conversion ratio (the kilogrammes of feed needed to produce a kilogramme of meat) would improve by 30 per cent, thereby saving 25 million tons of grain per year, and making grain imports almost unnecessary.

Because the country's land mass is so far north, the USSR has virtually no chance to grow natural protein in the form of soy (which requires a long growing season and lots of moisture). Imports of soy and soy meal have jumped from zero to 3 million metric tons annually from Brazil, Argentina and the U.S.A. in the past five years.

The U.S.S.R. now manufactures 1 million m.t./yr of proteins in industrial-scale fermenters, and hopes to double production during the current 1981-1985 Five-Year Plan, but a series of articles in the Communist Party daily Pravda recently noted that actual demand will be for 4 million m.t. by 1985, and adds that GLAV-MICROBIOPROM has not increased output appreciably in the past three years. In addition, the ruling Politburo noted at a special session in May that the Chief Administration "lacks a businesslike attitude ... and a sense of determination when it comes to fulfilling state production plans, and introducing new technology."

The basic Soviet technology for producing feed proteins by wood hydrolysis is now almost 16 years old. In this process, a cellulose sugar is enriched with mineral salts, and mixed in a fermenter with a yeast culture, yielding a powder of about 50 per cent protein content, plus vitamins. Most Soviet research in biotechnology has been devoted to finding a hydrocarbon raw material other than cellulose to use as a feedstock. The most promising appears to be paraffin, which the Soviets says they can reclaim as a by product of oil refining, or natural gas, a concept that is still in the experimental stage.

The Soviets also claim to have developed a mass-production process for making the amino acid lysine from molasses, which they obtain as a waste product from the country's sugarbeet plants. One litre of molasses yields between 40 and 70 g of lysine, and the Soviets hope to gear up for the manufacture of 17,000 m.t./yr of the acid by 1985. Soviet scientists are also experimenting with recovery of lysine from acetic acid. (Extracted from Chemical Engineering, 8 August 1983.)

Sweden

Swedish/Hungarian team to make food additive

Sweden and Hungary have set up a joint venture to produce a bacterial product its manufacturers say conserves fodder and prevents enteric diseases in piglets and calves. The firm, Monopharm Ltd., produces Lactiferm M 74 from Streptococcus faecium M 74 and conserves corn and grass silage by lowering the fodder's pH, thereby preventing the growth of some fungi and yeasts. If fed to young cows and pigs before their intestinal flora develop fully it can stop the growth of some pathogenic bacteria such as Salmonella, Shigella, and enterotoxic Escherichia coli. (Extracted from McGraw-Hill's Biotechnology Newswatch, 1 August 1983.)

PATENTS

Genetic patents

The Cohen-Boyer patent, for the basic process of genetic manipulation of bacteria, was thrust back into the limelight with the filing of an unusual petition seeking to reopen to public view the Patent Office's action on the pending application. Although patent applications are by law confidential, Stanford University and the University of California originally waived their right to secrecy, but later had the file closed to the public.

At the end of August the petition was filed to reopen the process to public view on the grounds that the only justification under the law for the secrecy of a pending application is protection of trade-secret material; by giving access to the file initially, Stanford had already exposed any such information.

New York's Columbia University was last week granted a patent that is widely viewed to be of sweeping importance to the future commercial production of proteins from mammalian cell cultures. The process patented was developed by Dr. Richard Axel and his colleagues. It enables two new genes to be simultaneously placed and activated in mammalian cells. One acts as a selectable marker, the other produces a protein that is under study or of commercial value.

The ability to introduce selectable markers is a key to the commercial use of mammalian cells in biotechnology applications. The markers allow easy isolation of the transformed cells.

Mammalian cells will probably be used to produce proteins that are toxic to bacteria or unstable in bacterial systems; mammalian cells offer in addition the substantial advantage of being easily induced to secrete their protein products. Thus, unlike bacterial cells, mammalian cells do not need to be destroyed to harvest the product. The ability to maintain stable lines of protein-producing cells also allows for manipulation of environmental factors to optimize production.

Foreign patent applications for the process are pending in Western Europe, Japan, Canada and Australia. Two additional US patents covering related work on gene promoters and amplified genes are also pending. (Source: Nature, 25 August 1983.)

Selection of recent patents

Assignee	Title	Patent Number/ Date Issued
Ajinomoto (Japan)	"Method for Producing L-Valine by Fermentation."	4,391,907 5 July 83
Damon Corp.	"Microcapsules Containing Viable Tissue Cells."	4,391,909 5 July 83
Ajinomoto (Japan)	"Method for Producing L-Glutamic Acid by Fermentation."	4,393,135 12 July 83
Upjohn	"Cloning Plasmid for Streptomyces."	4,393,137

Assignee	Title	Patent Number/ Date Issued
Yale University	"Method for Cloning Genes." (cDNA synthesis from mRNA mixture and identification of transformant containing desired gene).	4,394,443 19 July 83
Francis C. Szoka, Jr. and D.P. Papahadjopoulos (inventors)	"Method of Inserting DNA into Living Cells."	4,394,448 19 July 83
Ethyl Corp. Richmond, Va.	"L-Proline From Algae"	4,390,624 28 June 83
President and Fellows of Harvard College, Cambridge, Mass.	"Polypeptide Degrading Enzymes"	4,390,629 28 June 83
Talres Development (N.A.) N.V., Netherlands Antilles	"Immobilized Bacterial Ethanol Production"	4,393,136 12 July 83
E.N.I. Ente Nazionale Idrocarburi Rome, Italy	"New Source of Uricase"	4,398,485 21 June 83
Takeda Chemical Industries Ltd., Osaka, Japan	"Method for Producing Citric Acid"	4,389,484 21 June 83
Université Pierre et Marie Curie and Institut Pasteur Paris, France	"Prompt Diagnosis of Herpes Simplex Virus"	EPO 084 013 12 Jan. 83
Enzo Biochem, Inc. New York, N.Y. U.S.A.	"Rapid Local Treatment of Herpes"	EPO 080 032 26 Aug. 82
Imperial Chemical Industries PLC, London, United Kingdom	"Continuous Drying Apparatus for Single-Cell Protein"	EPO 080 277 1 Nov. 82
The Wistar Institute	"DNA Which Codes for Glycoprotein of ERA-Strain Rabies Virus."	4,393,201 12 July 83
Medical College of Georgia Research Institute	"Method for the Direct Analysis of Sickle Cell Anemia."	4,395,486 26 July 83
University of California	"Gene Transfer in Intact Mammals."	4,396,601 2 Aug. 83
Columbia University	"Processes for Inserting DNA into Eucaryotic Cells and for Producing Proteinaceous Materials."	4,399,216 16 Aug. 83
Shionogi & Co. (Japan)	"Semi-Synthesis of Human Insulin." (Converts animal to human insulin chemically and with Streptomyces enzyme).	4,400,465 23 Aug. 83
Immunex Corp.	"Process for Preparing Human Interleukin-2" (Produces IL-2 in human malignant T-cell line).	4,401,756 30 Aug. 83
Shionogi & Co. (Japan)	"Semi-Synthesis of Human Insulin." (Same as 4,400,465, above, but uses different bacterial enzyme).	4,401,757 30 Aug. 83

Assignee	Title	Patent Number/ Date Issued
Harvey Rubin (Inventor)	"Detection and Isolation of Glucagon mRNA Using a Synthetic Oligodeoxynucleotide." (Hybridization process isolates mRNA from pancreas cells).	4,401,759 30 Aug. 83
Ethyl Corp.	"Adenine Preparation." (Chemical synthesis of compound).	4,401,814 30 Aug. 83
University of Minnesota	"In vitro DNA-Protein Viral Assembly and Gene Cloning System." (Method for encapsulating genetic material).	4,403,035 6 Sep. 83
University of Iowa Research Foundation	"Genetic Reagents for Generating Plasmids Containing Multiple Copies of DNA Segments." (Plasmids with sequences containing 3 cleavage sites).	4,403,436 6 Sep. 83
Stichting Dr. Karl Landsteiner (Netherlands)	"Method of Culturing Hybridomas." (Endothelial cell growth factor).	4,404,279 13 Sep. 83
Steven Gillis (Inventor)	"Process for Preparing Murine Interleukin-2 in the Presence of Interleukin-1 from an Interleukin-2 Nonproducer Malignant Cell Line and Cell Line Therefor." (Cell line requiring IL-1 to stimulate IL-2 production).	4,404,280 13 Sep. 83
Transgene, S.A. (Paris, France)	"Vector for Catechol 2,3-Oxygenase"	EPO 086 139 27 Jan. 83
Zaidanhouzin Nagoyashi Kogyogijutsu Shinkokyokai (Aichi, Japan)	"Using Microorganisms for Biorecovery of Silver from Photo Film"	EPO 086 841 20 Aug. 82
Howard Florey Institute of Experimental Physiology and Medicine, (Parkville Victoria, Australia)	"Cloning Porcine Relaxin Gene"	EPO 086 649 11 Feb. 83
Bicgen N.V. Willemstad Curacao, (Netherlands Antilles)	"Preparing Human from Non-Human Insulin"	WO 83/02772 EPO 087 238 7 Feb. 83

(Sources: Genetic Technology News of August, September and October 1983. Adress: 158, Linwood Plaza, P.O. Box 1304, Fort Lee, N.J. 07024, USA and McGraw-Hills Biotechnology Newswatch of 1 and 15 August, and 17 October 1983.)

PUBLICATIONS

New journal

A new bi-monthly journal was launchrd by Elsevier Publications of Cambridge, UK, earlier this year. Trends in Biotechnology publishes concise, clearly presented articles on all aspects of biotechnology and provides a convenient and authoritive form of communication. Their aim is to present topical review and feature articles written by invited experts, which can be understood by all who have a serious interest in biotechnology, and to do so without over-simplification and trivialization. The editorial board is an international group of scientists and technologists whose expertise covers all aspects of biotechnology and who have links with universities, industry, government and research establishments.

New report on the biochip

Technical Insights Inc., have just issued a new report entitled Molecular Electronics: Beyond the Silicon Chip as the ninth in their Emerging Technology Series. The report provides an overview of today's molecular electronics, detailing problems, possible solutions, prospects and obstacles; it describes what each research group is doing and gives key names and addresses. Key patents already issued in this new and rapidly maturing field are referenced, as well as major literature references. At $227 per copy plus $20 for overseas buyers its not exactly cheap, but if one orders a copy and is not happy with the content and value of the report one can return it within a fortnight and get the money back. (Address: Technical Insights Inc., 158 Linwood Plaza, P.O. Box 1304, Fort Lee, N.J. 07024, USA.)

Booklet on India's biotechnology status

The Department of Science and Technology's National Biotechnology Board has recently issued a booklet on the status of biotechnology in India. In order to co-ordinate the R+D programmes in biotechnology in different national agencies and to provide appropriate direction for future R+D and training programmes, the government found it essential to formulate the long- and short-term plans in the field of biotechnology. The status report was compiled on the basis of information provided by the various agencies and gives an account of their ongoing research programmes. The booklet is available upon request from the National Biotechnology Board, Dept. of Science and Technology, Technology Bhavan, New Mehrauli Road, New Delhi 110016, India.

Recent UNIDO publications

The following is a selection of the papers presented at the Ministerial-Level Plenipotentiary Meeting on the Establishment of the International Centre for Genetic Engineering and Biotechnology, Madrid, Spain, 7 to 13 September 1983.

		Language
ID/WG.397/1	Report of the Selected Committee	E F S
ID/WG.397/2	Financial Matters Relating to the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	E F S
ID/WG.397/3	Practical Considerations of the Operation and Work Programme of the International Centre for Genetic Engineering and Biotechnology prepared by Burke K. Zimmermann	E F S
ID/WG.397/4	Draft Statutes of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/4/Add.1	Proposed Options for the Assessment of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/4/Add.2	Annotations to the Draft Statutes of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/5	Provisional Agenda	E F S
ID/WG.397/6	Organizational Matters prepared by the UNIDO Secretariat	E F S
ID/WG.397/7	List of Documents List of Background Documents	
ID/WG.397/8	Statutes of the International Centre for Genetic Engineering and Biotechnology	E F S

ID/WG.397/9	Report of the Ministerial-Level Meeting on the Establishment of the International Centre for Genetic Engineering and Biotechnology Provisional List of Participants	E F S

List of documents for the Second Consultation on the Pharmaceutical Industry (Budapest, Hungary, 21-25 November 1983)

ID/WG.393/5	Progress Report	E F S R C

Issue and background papers

ID/WG.393/6	Contractual Arrangements for the Production of Drugs - Issue Paper	E F S R C
ID/WG.393/1	- Items which could be incorporated in contractual arrangements for the transfer of technology for the manufacture of those bulk drugs/intermediates included in UNIDO's Illustrative List	E F S

Issue and background papers (cont'd)

ID/WG.393/4	- Items which could be included in contractual arrangements for the setting up of a plant for the production of bulk drugs (or intermediates) included in UNIDO Illustrative List	E F S
ID/WG.393/3	- Items which could be included in licensing arrangements for the transfer of technology for the formulation of pharmaceutical dosage forms	E F S
ID/WG.393/7	Contractual Arrangements for the Production of Drugs - Background Paper	E F S R C
ID/WG.393/8	Availability, pricing and transfer of technology for bulk drugs and their intermediates - Issue Paper	E F S R C
ID/WG.393/9	Availability, pricing and transfer of technology for bulk drugs and their intermediates - Background Paper	E F S R C
ID/WG.393/10	The development of drugs based on medicinal plants - Issue Paper	E F S R C
ID/WG.393/11	The development of drugs based on medicinal plants - Background Paper	E F S R C
ID/WG.393/12	The manufacture of vaccines in developing countries - Issue Paper	E F S R C
ID/WG.393/13	The manufacture of vaccines in developing countries - Background Paper	E F S R C

Reference papers

ID/WG.393/17	Relevant topics to be taken into account in the preparatory phase of technology transfer arrangements for the production of pharmaceuticals	E
ID/WG.393/16	Summary of industrial property protection on pharmaceuticals in developing countries	E F S
ID/WG.393/	Multipurpose plant for the production of UNIDO's List of Essential Drugs based on raw materials and intermediates	
ID/WG.393/2	Directory of Sources of Supply of 26 Essential Drugs, their chemical intermediates and some raw materials	

New report on the biochip

Technical Insights Inc., have just issued a new report entitled Molecular Electronics: Beyond the Silicon Chip as the ninth in their Emerging Technology Series. The report provides an overview of today's molecular electronics, detailing problems, possible solutions, prospects and obstacles; it describes what each research group is doing and gives key names and addresses. Key patents already issued in this new and rapidly maturing field are referenced, as well as major literature references. At $227 per copy plus $20 for overseas orders its not exactly cheap, but if one orders a copy and is not happy with the content or value of the report one can return it within a fortnight and get the money back. (Address: Technical Insights Inc., 158 Linwood Plaza, P.O. Box 1304, Fort Lee, N.J. 07024, USA)

Booklet on India's biotechnology status

The Department of Science and Technology's National Biotechnology Board has recently issued a booklet on the status of biotechnology in India. In order to co-ordinate the R+D programmes in biotechnology in different national agencies and to provide appropriate direction for future R+D and training programmes, the government found it essential to formulate the long- and short-term plans in the field of biotechnology. The status report was compiled on the basis of information provided by the various agencies and gives an account of their ongoing research programmes. The booklet is available on request from the National Biotechnology Board, Dept. of Science and Technology, Technology Bhavan, New Mehrauli Road, New Delhi 110016, India.

Recent UNIDO publications

The following is a selection of the papers presented at the Ministerial-Level Plenipotentiary Meeting on the Establishment of the International Centre for Genetic Engineering and Biotechnology, Madrid, Spain, 6 to 13 September 1983:

		Language
ID/WG.397/1	Report of the Selected Committee	E F S
ID/WG.397/2	Financial Matters relating to the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	E F S
ID/WG.397/3	Practical Considerations for the Operation and Work Programme of the International Centre for Genetic Engineering and Biotechnology prepared by Burke K. Zimmerman	E F S
ID/WG.397/4	Draft Statutes of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/4/Add.1	Proposed Options for the Assessment of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/4/Add.2	Explanatory Notations to the Draft Statutes of the International Centre for Genetic Engineering and Biotechnology prepared by the UNIDO Secretariat	A C E F S R
ID/WG.397/5	Provisional Agenda	E F S
ID/WG.397/6	Organizational Matters prepared by the UNIDO Secretariat	E F S
ID/WG.397/7	List of Documents List of Background Documents	
ID/WG.397/8	Statutes of the International Centre for Genetic Engineering and Biotechnology	E F S

UNIDO/IS.388	Water use and effluent in the Pharmaceutical Industry	E F S
UNIDO/IS.402	Prospects for production of vaccines and other immunizing agents in developing countries	
ID/WG.393/15	The need of drug policies	E
ID/WG.393/14	Industrial profiles of pharmaceutical production units for formulations and bulk drugs	E
UNIDO/PC.76	Report of the Meeting about Technical Cooperation Among Developing Countries (Tunis, September 1983)	E F S

Two papers presented at the 8th Meeting of Heads of Technology Transfer Registries, held at Caracas, Venezuela from 17 to 21 October 1983.

ID/WG.405/5	Restrictive clauses in licensing agreements in the pharmaceutical industry by J. Cieslik	E
ID/WG.405/7	Monitoring of technology transfer agreements by regulatory agencies - an overview of policies and issues	E

MEETINGS

Date	Title
4-6 December 1983	International Seminar on 'Modelling and Simulation for Safety and Risk Assessment', London, UK, (Dr. A.J. Jouhar, Information Transfer International, P.O. Box 62, Beaconsfield, Bucks HP9 2NY, UK.)
12-21 December 1983	XV International Congress of Genetics, New Delhi, India. (Dr. M.S. Swaminathan, Planning Commission, Yujana Bhavan, New Delhi 110001, India.)
14-15 December 1983	Symposium on Scaling-Up Bioprocesses from Laboratory to Commercial Stage. Columbus, OH. Contact: Ruth Anne Gibson, Battelle Columbus Laboratories, 505 King Ave., Columbus, OH 43201, 614-424-5327.
16 December 1983	Cannabinoids - their possible therapeutic uses? London, UK. (Ms. Barbara Cavilla, c/o Institute of Biology, 20 Queensberry Place, London SW7 2DZ, UK.)
20-21 December 1983	Molecular Variants of Proteins - Biosynthesis and Clinical Relevance, London, UK. (Prof. P.N. Campbell, Director, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, Mortimer Street, London W1P 7PN, UK.)
9-11 January 1984	The Chilton Conference on Inositol and Phosphoinositides, Dallas, Tx, USA. (Dr. J.E. Bleasdale, Division of Lipid Research, Dept. of Biochemistry, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235, USA.)
16-20 January 1984	Advances in Gene Technology: Human Genetic Disorder, Florida, USA. (Miami Winter Symposia, P.O. Box 016129, Miami, Florida, USA.)
29 January - 3 February 1984	Recovery of Biological Products, Sea Island, Ga. Engineering Foundation Conferences, 345 E. 47th St., New York, NY 10017.
6-10 February 1984	Protein Structure and Function, Victoria, Australia. (Dr. Richard J. Simpson, St. Vincent's School of Medical Research, 41 Victoria Parade, Fitzroy, 3065, Victoria, Australia.)

Date	Title
13-16 February 1984	Structure and Expression of Eukaryotic Genomes, Victoria, Australia. (Dr. G. Miklos, Research School of Biological Sciences, Australian National University, GPO Box 4, Canberra City, 2601, Australia.)
16-18 February 1984	The Nucleus, Melbourne, Australia. (Dr. J. Cammakaris, Department of Genetics, Melbourne University, Parkville, 3065, Victoria, Australia.)
19-25 February 1984	VII International Biotechnology Symposium, New Delhi, India. (Prof. T.K. Ghose, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India.)
20-22 February 1984	International Symposium on HPLC in the Biological Sciences, Melbourne, Australia. (Mrs. S.R. Tregellas, St. Vincent's School of Medical Research, Victoria Parade, Fitzroy, 3065, Victoria, Australia.)
20-22 February 1984	Tumor Markers in Cancer Control, Vienna, Austria. (Humor Tumor Markers Conference, AMEX POB No. 790459, Dallas, TX 75379, USA.)

(Sources: TIBS, September 1983. McGraw-Hill's Biotechnology Newswatch, 19 September 1982 and Genetic Technology News of August and September 1983. Address: 158, Linwood Plaza, P.O. Box 1304, Fort Lee, NJ 07024, USA.)

REPRINTED ARTICLES

Bright future for the biological fix by Dr. Peter Cheetham, Philip Lyle Memorial Research Laboratory, Tate and Lyle, Reading.

(Reprinted from Spectrum 1983/No. 184/1. Copies may be obtained in French and Spanish from any British Embassy, High Commission, Consulate or the Central Office of Information, Hercules Road, London SE1 7DU, England.)

Putting enzymes to practical use is a technology still in its infancy. Properly exploited, they will make an enormous impact on the world problems of disease, malnutrition, energy and pollution. Especially promising is the developing technique of fixing them in some way to solid particles, when they become known as immobilized enzymes. In that form they are more stable, easier to handle and can be used again or used continuously. Moreover, recent advances in genetic engineering will make these versatile and hitherto natural catalysts available cheaply, abundantly and in improved versions.

Enzymes are protein molecules, biological catalysts that play a vital part in a vast number of natural processes. They can be harnessed to speed up a very large range of chemical reactions, allowing them to be carried out at lower temperatures and thereby saving a great deal of energy. They are extremely versatile and are stereospecific, that is, each kind of enzyme recognizes and works on molecules of one specific spatial structure. The molecules, or the material on which they act, is called the substrate. Several thousand enzymes with different substrate specificities are known already and it is certain that a vast number still remain to be discovered.

Enzymes have many advantages over chemical catalysts or fermentation processes. The advantages include the very wide range of reactions that enzymes can catalyse; the plentiful supply of many enzymes that can be obtained from microbes or from certain animals and plants; the precise substrate specificities of most enzymes and, most remarkably, the very fast stereospecific reactions that they make possible under mild conditions of temperature and pressure and in the cheapest available solvent, water.

There is, however, one big disadvantage. Enzymes are usually unstable enough to lose their activity rapidly, especially when they are used at high temperatures or in the presence of organic solvents. So one way of defining enzyme technology is to say that it attempts to exploit the advantageous properties of enzymes for industrial, medical, analytical and other applications while overcoming in some way their inherent disadvantages.

Fermentation and, to a lesser degree, the use of soluble enzymes have long been applied commercially, for example in the antibiotics and starch-processing industries. Recently, immobilized enzymes and cells have begun to make their mark in the food, beverage and pharmaceutical fields. One important reason why enzymes are especially useful is that they do not need such complex substrates as do fermentation processes, in which it is necessary to support the growth of microbial cells. Purification of the products is simpler, too: fewer wastes, and less of them, are produced and enzymic reactions can go on under conditions that would normally inhibit cell growth.

Restricting movement

Immobilization means restricting the free movement of the enzymes or cells, usually by attaching them to or trapping them within an inert, solid support, though soluble immobilized enzymes can be produced and are especially useful for treating macromolecular solid substrates. Immobilizing the enzymes or cells allows them to be used again or used continuously. It also ensures that they do not contaminate the product, for they can be more easily filtered off or collected by sedimentation or by using a centrifuge. Sometimes immobilization has the effect of concentrating the enzyme so that the reaction can take place in less time or in a more compact container than when the soluble enzyme is used. Moreover, the technique may improve the stability of enzymes and cells during operation and in their response to temperature, so that they can be used in ways that are kinetically and logistically more convenient. Nevertheless, it often means that a great deal of enzymic activity is lost and, of course, immobilization is an extra step in an industrial process and may be expensive and difficult to perform on a large scale.

Many ways of immobilizing enzymes and cells have been developed in the laboratory but very few have been implemented successfully on a commercial scale. The biochemical support or the reagents may be expensive or toxic; immobilization may be difficult to achieve on a large scale or, sometimes, the immobilized biocatalysts are not mechanically suitable.

The various techniques may be classified into the following main groups:

Absorption of the enzyme or cell onto oppositely-charged support particles.
Encapsulation inside semi-permeable membranes.
Co-valent bonding to chemically-activated supports.
Aggregation or cross-linking of the cells or enzymes themselves.
Entrapment in polymeric matrices; gel supports prove to be especially favourable for immobilizing cells of plants and even of animals.

Selecting the best way of immobilizing individual types of enzyme or cell is still empirical. The aim is to find a simple, cheap and safe way of yielding preparations that have the greatest possible enzyme activity, yet are stable enough for the process for which they are intended.

The level of activity depends upon several features of the immobilization process itself and others that affect the access of the substrate molecules to the enzymes.

In many instances it is preferable to use one or more enzymes still associated with their parent cells. This is often advantageous when it would be difficult or expensive to extract, purify or concentrate the enzyme in an active force and when multi-step reactions using two or more enzymes working together are required.

Immobilized cells are especially useful in synthetic reactions that consume energy. In such applications it is often necessary to supply nutrients to the immobilized cells so that the reaction can proceed in a way that allows some growth of the cells within the supporting material. But complex or synthetic reactions are usually difficult to perform in conventional and cheap ways, and the concentration of enzyme in immobilized cell preparations is usually low. So far, all commercially successful applications of immobilized enzymes use only one kind of enzyme to carry out a relatively simple hydrolysis, oxidation or isomerization reaction and the enzymes are normally extracellular, that is, secreted from their parent organism. In these cases it is obviously better to immobilize the enzymes themselves instead of the cells that contain them.

Enzyme reactors

The container for an enzyme-catalysed reaction, with its associated pumps, stirrers and sampling and monitoring devices is known as an enzyme reactor. Its job is to produce a specified substance from specific substrates at a set rate and as cheaply as possible. They might use soluble or immobilized enzymes; they work at lower temperatures than chemical reactors, and the product of the reaction does not catalyse the reaction itself as in fermentation.

An efficient enzyme reactor should use as small an operating volume as possible to produce a high yield of product in a concentrated form. So it is very desirable to use high concentrations of substrate and high operating temperatures, and to achieve the greatest possible degree of conversion of substrate to product.

The main types are stirred and column reactors. Stirred reactors are preferred when the need is for good mixing of their contents, addition of gases or strict control of pH. Column reactors have the advantage of greater productivity and they keep inhibition of the enzyme reaction by products to a minimum.

Industrial uses

Immobilized enzymes and cells are chiefly used as heterogeneous catalysts in the food and pharmaceutical industries. They are also used as analytical devices such as so-called enzyme electrodes, and in medicine for removing poisons from blood. Potential uses are in energy generation, disposal of waste and its detoxification, and in many other fields. Such applications should be encouraged by recent advances in genetic engineering that promise to make any enzyme available cheaply and abundantly. In particular, the modification of enzyme properties being pioneered in collaboration between the UK Medical Research Council's Laboratory of Molecular Biology, at Cambridge, and the Chemistry Department of Imperial College, London, will be a big advantage.

The very first report of an immobilized enzyme came in 1908; it described invertase, which splits sucrose into glucose and fructose, immobilized onto charcoal. Invertase was also the first immobilized enzyme known to be employed by industry, for it was used in the UK by Tate and Lyle during World War II to form syrups when the preferred reagent, sulphuric acid, was unavailable.

The longest-established example of immobilized-enzyme technology is in Japan, where the Tanabe Seiyaku company has employed Aspergillus oryzae L-amino acid acylase, immobilized to a substance known as DEAE-Sephadex, for producing L-amino acids from racemic mixtures. The immobilized enzyme discriminates between the two optically-active forms of the acylated amino acid and has been in commercial use for about ten years.

Other systems being operated in a large scale in that country include processes for producing aspartic and malic acids. In both instances the enzymes are immobilized in gellified particles complete with their parent microbial cells. The immobilized particles are packed into columns, substrate is pumped through and the product is eluted. About 600 and 180 tonnes of the respective amino acids are produced in this way each year for use in foods and medicine.

So far, the most successful commercial example of immobilized-enzyme technology is immobilized glucose isomerase. Its preferred substrate is xylose, and it is particularly suitable for industrial use because it requires no other 'co-factor' to work with it, and is relatively stable when used at raised temperatures (of about 60°C) to keep microbial contamination to a minimum. A good source of the enzyme is Bacillus coagulans. Whether extracted from the cells or still within them, the enzyme is used in large, packed column reactors to convert glucose syrups, derived from corn starch, into a glucose-fructose syrup. The syrup contains up to 55 per cent fructose and is sweeter than glucose syrup or comparable sucrose syrups, is not coloured and does not contain the side products generated during chemical processes. This process has developed rapidly in the last ten years, notably in the USA, and product has displaced sucrose from many of its traditional markets, for example as a sweetener in soft drinks.

Semi-synthetic penicillin

Several academic groups in the UK have pioneered the use of immobilized enzymes and the development of enzyme technology. The efforts of a research group at University College, London, have led to the use by Beechams Pharmaceuticals of immobilized penicillin acylase to convert penicillin G (benzyl penicillin) into 6-amino-penicillanic acid (6APA), which is useful as the precursor for chemically synthesizing a whole range of semi-synthetic penicillin antibiotics. All of the UK's semi-synthetic penicillins are now made in this way. The enzyme, which is obtained from E. Coli, is soaked into polymer beads, cross-linked into place and used in batches in large stirred vessels. Alkali is added continuously, to neutralize the acid produced during the enzyme-catalysed reaction. The advantages over comparable chemical processes include a higher yield by keeping side products to a minimum.

Several other processes in the UK have been developed a long way towards successful commercial application. One good example is the immobilization of amyloglucosidose (glucoamylase) by Tate and Lyle: the enzyme is immobilized onto bone-char as a gel of enzymically-active protein by simultaneous precipitation with acetone and cross-linking with glutaraldehyde. The immobilized enzyme is used in large columns in starch-processing factories for the rapid and almost complete saccharification of pre-thinned starch syrup to glucose. The productivity of this system is greatly superior to that obtainable from earlier processes that use soluble amyloglucosidase.

Pilot trials

Tate and Lyle have also successfully completed pilot trials recently on forming isomaltulose from sucrose, using columns of immobilized bacterial cells. One important feature of the process is the way the enzyme activity of the whole cells was stabilized through immobilizing them in alginate gel, using concentrated pure sucrose as substrate, and by attaining a high degree of conversion of sucrose into isomaltulose. The enzymes have a half-life of one year.

Unilever are using lipases immobilized to celinte to convert glycerides to more valuable forms, including fats that could serve as replacements for cocoa butter. The substrate is dissolved in an organic solvent such as petroleum ether saturated with water and is passed through a column of the immobilized enzyme.

The UK Milk Marketing Board are carrying out trials on lactase (β -galactosidase), immobilized to particulate silica beads, to hydrolyse the lactose in whey and similar dairy products to make sweeter, more soluble and more easily digested syrups.

Other promising work includes the use of enzymes immobilized to magnetic supports as a means of recovering them more easily; using immobilized yeast and bacteria to form ethanol; the development of enzyme-based sensors and biofuel cells, and the immobilization of plant cells - especially cells that are able to fix atmospheric nitrogen.

Obviously, the technology of putting enzymes to practical use is still in its infancy. Comparatively few convincing commercial successes have been achieved and very many important problems remain to be solved. They include the use of water-immiscible and insoluble substrates; use of plant and animal cells and membrane-bound enzymes as catalysts; use of co-immobilized enzymes and cells, and the ability to regenerate co-enzymes to perform complex, synthetic reactions. If these challenges can be overcome, the versatile and remarkable catalytic resource that can be harnessed will make an immense impact on great world problems such as disease, malnutrition, energy and environmental pollution.

* * * * * * * * *

The following article, which appeared in Volume 1, No. 1 of Trends in Biotechnology (Elsevier Publications, Cambridge, UK) was written by Professor Irving Kayton, Director of the Patent Law Program, The George Washington University and President of Patent Resources Group, Inc., of Washington DC, USA. In consideration of the considerable interest in patenting of copyright protection, we thought our readers may be interested in knowing more about the US Law in this respect. The article was adapted from the George Washington Law Review (1982), 50, 191-218, which presents more detailed information on the legal and constitutional issues.

Does copyright law apply to genetically engineered cells?

The words you are now reading are a work of authorship protected by copyright laws from unauthorized reproduction. Genetic engineers may be surprised to learn that their manipulations of genetic information within cells can also be works of authorship which US copyright law protects from unauthorized reproduction.

The idea that genetic engineers can make use of copyright law may be startling, but a close examination of the US Copyright Act of 1976 indicates that this law could have an immense impact on biotechnological research, development and production. The central arguments of this article are that virtually all original works of a genetic engineer are copyrighted automatically when they are created; that scientists generally can enforce copyrights; and that those copyrights may provide more effective protection than patents or trade secrets.

Advantages of copyright protection

What is the value in copyrighting genetic information? Under certain circumstances, from a practical as well as legal viewpoint, copyright protection may be the only or the most effective way an author can protect a valuable genetic work. Furthermore, copyright protection offers unusually attractive remedies and long life.

The range of benefits open to a copyright owner is truly impressive. The Justice Department may prosecute anyone who wilfully infringes a copyright for commercial advantage or private gain. In addition, upon conviction a court may order the destruction of all 'implements, devices, or equipment used in the manufacture' of infringing copies. The convicted copyright infringer is not only out of the copying business, but also out of any microbiological business for want of an industrial plant. A copyright owner can recover both his own actual damages and the infringer's profits.

While patent protection exists for seventeen years and trade secrets last for as long as they can be kept secret, copyright exists from the time of the work's creation until fifty years after the death of the author. Most importantly, the copyright of works made for hire, e.g. by an employee of a corporation, endures for one hundred years after the creation of the work or seventy-five years after publication of the work, whichever is earlier. Thus, copyrights last much longer than patents and impose none of the problems or expense of safeguarding a trade secret.

Copyright protection under the 1976 Act

We now come to the central questions: can genetically engineered organisms be copyrighted and, if so, can the copyright be enforced? The Act of 1976 provides copyright protection from the moment a 'literary work' is created. A work is created when it is 'fixed in a tangible medium of expression'. In turn, a work can be fixed in any 'form, manner, or medium as long as it is sufficiently permanent or stable to permit it to be perceived, reproduced, or otherwise communicated for a period of more than a transitory duration'. The act of creation may take place in the quiet and secrecy of a private laboratory. Property rights immediately accrue, which may be enforced once the copyright is registered.

Libraries of spliced DNA fragments and cultures of engineered cells with a foreign DNA sequence are certainly fixed in permanent and tangible media of expression. Moreover, genetic works are stable enough to be perceived and reproduced. Reproduction, in the case of the cell, is the entire point of genetic engineering and perception is feasible by DNA sequencing. Under the 1976 Act, perception may be direct or 'with the aid of a machine or device'.

The crucial question now is whether genetically engineered cells can be considered to be 'literary works'. At first sight this seems a highly unlikely supposition, but the definition of a literary work is much wider than most scientists and engineers realize. It includes 'works ... expressed in words, numbers, or other verbal or numerical symbols or indicia, regardless of the nature of the material objects ... in which they are embodied'. A digital computer program or data base is a literary work; it can be expressed in 'indicia', such as magnetic impulses or holes in a punch card; moreover the tapes or cards enable the information to be 'fixed in ... tangible medi(a) of expression'. Similarly, genetically engineered works are expressed in 'indicia', the nucleotides of DNA. Genetic works are fixed in tangible media of expression, such as cells and cultures of cells. The term 'gene library' may be more apposite than genetic engineers have realized.

Computers output their operations in a number system that is usable by people. Within the computer the binary notation is converted to decimal just prior to printout. In a cell, the genetic information contained in DNA is also transferred into something usable - proteins (which effectively represent a different number system than the DNA triplets). Thus, like a computer program, a genetic work uses indicia to transfer information. Whether the genetic scientist or engineer takes pen in hand, invents something patentable or does both, he apparently authors a literary work when he applies the techniques of recombinant DNA to create original DNA sequences.

Some may argue that recombinant DNA work is not sufficiently original to be copyrightable. Every DNA fragment combined with any other and every plasmid introduced into a host cell already exist in nature. This argument is, however, analogous to one suggesting

that this article is not original because the words the author is stringing together are all well-known and appear in standard dictionaries. Moreover, the 1976 Act states that a compilation of pre-existing materials may itself be original: 'A "compilation" is a work formed by the collection and assembling of preexisting materials or of data that are selected, coordinated, or arranged in such a way that the resulting work as a whole constitutes an original work of authorship'. If each DNA fragment or plasmid wants for originality, each combined by man with others does not. At the very least, splicing of DNA fragments and introduction of DNA materials into a host cell are original and often novel compilations.

Registration of copyright

Although copyright protection 'subsists' in a work as soon as it is created, that protection is not legally enforceable until the copyright is registered. For an infringement which took place before registration the copyright owner can recover actual damages but cannot generally obtain statutory damages or attorney's fees. Two registration requirements are of interest because of the peculiar nature of genetically engineered works: affixing a copyright notice to the work when it is published, and depositing two copies of a published work or one copy of an unpublished work. The 1976 Act defines 'publication' quite broadly. Publication includes a mere offer to distribute copies to individuals for purposes of further distribution.

Ordinarily, a copyright notice must appear on the work when published. One significant exception exists, however, to this requirement. A copyright notice need not be affixed to a published work if it cannot be visually perceived. For example, a movie sound track can be protected without affixing a notice of copyright to the film. Like movie sound tracks, virtually all genetic works of interest are not visually perceivable. As a general proposition, therefore, a notice of copyright does not have to be imprinted upon a genetic work. To provide extra security, a copyright owner could affix a copyright notice to the base of the petri dish, or use a fine pipette to deposit fluid containing the engineered cells on agar in a pattern approximating a notice of copyright!

Although the 1976 Act requires the copyright owner to deposit two copies of the best edition of the work, it does not preclude him from depositing a whole petri dish with a million cells. There seems to be no need to ensure that the works remain alive after deposit at the Copyright Office. Most importantly, the statute permits the Register of Copyrights to exempt any category of materials from the deposit requirement and thus the 1976 Act provides the Copyright Office with sufficient flexibility to cope with the registration of genetic works.

Infringement of copyright

Copying or reproduction of the copyrighted work is an indispensable ingredient of copyright infringement; if someone other than the copyright owner subsequently originates the very same work as the one copyrighted, the subsequent author is free to: (a) copy and reproduce his own work, even though it is identidal to the earlier copyrighted work; (b) obtain a copyright on his own work, although it is identical to the copyrighted work; and (c) prevent others, including the earlier author, from making copies of or reproducing his work.

If the work is long and detailed, and identical to an earlier one, for example, Shakespear's Macbeth, the probability is virtually zero that the subsequent author originated, rather than copied it. For this commonsense reason, evidence of copying may be circumstantial and yet very persuasive. If the author/inventor includes in the DNA compilation one or more redundant gene fragments that are totally unnecessary and unrelated to the function of the DNA compilation these so-called 'housemarks', if found in the plagiarist's work, will be virtually conclusive proof of copying. In the case of genetic engineering, it is noteworthy that all the DNA sequences which code for a specific protein must be at least two-thirds identical, even if the plagiarist makes full use of the redundancy in the genetic code.

Perspectives in protection

Most molecular biologists are, or until recently have been, employed by universities, non-profit research institutions, or governments. In the past, these employers rarely restricted the scientists' right to publish their work. The situation has changed rapidly. Universities recognize that gold mines as well as test tubes and Ehrlenmeyer flasks are scattered around their microbiological laboratories and that the gold is recoverable only by perfecting their property rights. Moreover, business arrangements between research institutions and private corporations always centre on effectively protecting innovation.

The net result is massive ambivalence on the part of the scientists and university and research administrators concerning the right of scientists to publish immediately and freely. Half of their being says publish immediately, and half says be sure to obtain protection so that funds for further research will be forthcoming.

Fortunately, immediate publication and copyright protection are completely compatible. Since the creation of a genetically engineered work itself generates the protection provided by copyright, publication of research findings on the day they are made will in no way impair copyright protection of those results.

One may reasonably ask whether copyright protection, with its attractive remedies and length of protection, is too great a reward for innovation in genetic engineering. Should the market have to pay that high a price simply because of governmental fiat? Would not the same quantity and quality of innovation be forthcoming with fewer and smaller legislated rewards? These questions have no easy answer, but surely a genetic engineer deserves at least the same reward as that given to the author of a detective story, cookbook, rockmusic record or computer programme.

Perhaps more to the point is that copyrights (and patents) teach the world the state of the art rather than hiding it as occurs with secrecy. Those of skill are then enabled either to take the next step beyond (and then negotiate for an exchange of rights) or to innovate around the protected work so as not to have to pay for its use. In either event, society benefits.

Reference

The Copyright Act of 1976, Title 17 of the United States Code, Paragraphs 101-810 (1976 and Supplement IV 1980)

Assistance required: Assistance wanted

We are including this request again because last time half of the item somehow disappeared in the works.

One of our readers has asked whether any research institution or company can supply them with information on the following:

(a) Research underway in the field of tissue culture;
(b) Institutions working on the subject;
(c) Scientists willing to help Colombia and its Association in R+D projects in the fields of genetic engineering and tissue culture;
(d) The Asociacion Colombiana de Cultivo de Tejidos Végetales (ACUTEV) has a project on the subject of tissue culture techniques and is looking for ideas, scientists and consultants.

If any of our readers has something to offer on the matter please contact Dr. Ernesto Velez Koppel, President, ACUTEV, Carrera 6, No. 77-51, Bogota, Colombia.

FOR NEW SUBSCRIBERS ONLY:

UNITED NATIONS NATIONS UNIES

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO MAILING LIST QUESTIONNAIRE

Genetic Engineering and Biotechnology Monitor
(Code: 503)

UNIDO MAILING LIST, INDUSTRIAL INFORMATION SECTION,
P.O. BOX 300, A-1400 VIENNA, AUSTRIA

	Type or print clearly (one letter per box) and leave a space between each word
NAME (underline family name)	
TITLE OR POSITION	
ORGANIZATION	
STREET AND No. (or P.O. Box)	
CITY AND STATE OR PROVINCE	
COUNTRY	

PLEASE DO NOT WRITE IN THESE SPACES

S/A ☐ DISP. CODE ☐ COUNTRY ☐☐☐

CITY ☐☐☐☐☐☐☐☐☐☐☐☐☐