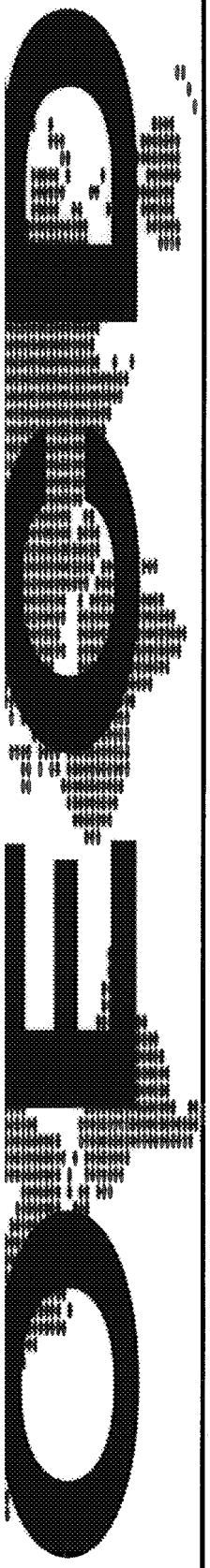

STI REVIEW No. 19

SCIENCE TECHNOLOGY INDUSTRY

Special Issue on Biotechnology

No. 19

STI REVIEW

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Pursuant to Article 1 of the Convention signed in Paris on 14th December 1960, and which came into force on 30th September 1961, the Organisation for Economic Co-operation and Development (OECD) shall promote policies designed:

- to achieve the highest sustainable economic growth and employment and a rising standard of living in Member countries, while maintaining financial stability, and thus to contribute to the development of the world economy;
- to contribute to sound economic expansion in Member as well as non-member countries in the process of economic development; and
- to contribute to the expansion of world trade on a multilateral, non-discriminatory basis in accordance with international obligations.

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FOREWORD

Prepared by the OECD Directorate for Science, Technology and Industry, the *STI Review,* published twice yearly, presents studies of interest to science, technology and industry policy makers and analysts, with particular emphasis on cross-country comparisons, quantitative descriptions of new trends and identification of recent and future policy problems. Because of the nature of OECD work, the *STI Review* explores structural and institutional change at global level as well as at regional, national and sub-national levels. Issues often focus on particular themes, such as surveys of firm-level innovation behaviour and technology-related employment problems.

This is the first *STI Review* on the subject of biotechnology. But what is biotechnology? One of the authors in this issue proposes a definition for his own chapter. However, the *Review* will make no such efforts beyond citing an earlier and useful OECD definition, influential in both academic and government circles: "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (OECD, *Biotechnology: International Trends and Perspectives,* Paris, 1982). Rather than a definition, this issue of the *Review* presents a picture of biotechnology in action.

The views expressed in this publication do not necessarily reflect those of the Organisation or of its Member countries. The *STI Review* is published on the responsibility of the Secretary-General of the OECD.

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INTRODUCTION: ON THE PERVASIVENESS OF BIOTECHNOLOGY

This is the first *STI Review* on the subject of biotechnology. But what is biotechnology? One of the authors in this issue proposes a definition for his own chapter. However, the *Review* will make no such efforts beyond citing an earlier and useful OECD definition, influential in both academic and government circles: "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (OECD, *Biotechnology: International Trends and Perspectives,* Paris, 1982). Rather than a definition, this issue of the *Review* presents a picture of biotechnology in action.

A quick survey of the titles of the seven contributions could create the impression of a haphazard collection of unrelated articles, throwing together humans and animals; healthy and sick; genes, viruses and microbes; plants, crops and trees; water, climate and deserts.

But the incoherence is only apparent. As biotechnology applications grow in number, more and more aspects of life and nature, of economy and society are affected by the new knowledge, and by the products and processes which have grown out of a scientific revolution. The starting points of this historic development can be identified and dated, the "endpoints" cannot – there is no end to mankind's continuous search for deeper understanding and more intelligent use of nature. That scientific revolutions cause major modifications and upheavals in other realms of life is not new. This will happen and is already happening, also for biotechnology; this issue of the *STI Review* identifies some consequent policy issues.

The structure and contents of the *Review* can be summarised as follows:

Human health

- "Biotechnology and the New Revolution in Health Care and Pharmaceuticals" (E. Ronchi). In a wide-ranging overview, Ronchi reviews the major contributions of biotechnology to the recent, extraordinary progress in biomedical research and in its applications, and points to many policy questions raised by this progress.

- "Intellectual Property in Genome Research Results" (J. Straus). A leading legal expert reviews intellectual property as one of the key problem areas that will affect – positively or negatively – all future progress in biotechnology, particularly its applications to human health. Industrialists tend to view such protection as a crucial condition for investment in research, and hence for successful biotechnology development. Commenting on an issue which has recently led to much controversy, often ranging widely beyond legal questions, Straus argues the case for intellectual property protection.

Food Crops and Animals

- "A Perspective on Crop Biotechnology for the First Half of the 21st Century" (W. de Greef). New biotechnology could bring major productivity growth to the world's main crop plants, and to the main crop-producing regions. Productivity increases will be necessary to address the world's long-term food needs in environmentally acceptable ways. de Greef reviews the great economic potential of the technology and mentions some of the stumbling blocks which must be overcome.
- "Animal Biotechnology" (L.M. Houdebine). Houdebine presents a comprehensive review of the potential of genetic modification in animals, both for agricultural and health (human or animal) purposes. The author recognises the opposition of an important section of public opinion to some, if not all, uses of transgenic animals, but nonetheless argues for further progress in animal biotechnology.

Environment

- "Environmental Biotechnology for the Millennium" (M. Griffiths and P. Hesselink). The authors show the ever-increasing options that biotechnology is opening up to remediate and prevent environmental pollution. Its "green" potential makes biotechnology an essential component of any "sustainable" civilisation.
- "Biotechnology and Water Conservation" (E. Aceves and C. Gobbee). Water, or lack of it, is the most critical of the sectoral environmental challenges, and that most likely to lead to economic and political disruptions. Aceves, an expert from Mexico, a country with severe water problems, reviews how biotechnology can help to conserve water, through pollution prevention, more efficient agricultural water use, and better adaptation to desert conditions.

– "Bioremediation: A Challenge to Education and Training" (R. Atlas). Among the radical transformations brought about by scientific revolutions are those of the educational and training systems. These transformations may take a generation. Atlas analyses some of the educational implications of the development and diffusion of bioremediation technologies.

The authors of these seven chapters are objective, but not "neutral". All have made important professional contributions to biotechnology as scientists, experts, teachers, managers or government officials. They underline its importance to economy and society, and point to problems which need to be addressed; however, they do not give equal time or space to the fears expressed by some of the interested public, nor to the ideological opposition of particular groups and parties to biotechnology and gene technology, its most powerful tool.

Governments have responded to these fears, and to legitimate concerns, by pursuing at both the national and international levels the most elaborate safety discussions ever to have accompanied the emergence of a new technology. They have put in place regulatory regimes for the products or processes of biotechnology, and are now endeavouring to harmonize these regulatory regimes internationally, while adapting them in the light of experience.

That safety considerations are little reflected in this issue of the *STI Review* does not mean that their great importance, particularly in the recent past, is ignored. However, the decision was taken to focus on scientific and technological trends, on economic potential, and on the future. The great controversies over the safety of DNA recombination *per se* are more a thing of the past than of the future – which is not to belittle the value of ongoing work related to the application and harmonization of safety rules in the many sectors where these are important.

In emphasizing the scientific, technological and economic aspects of biotechnology, this issue of the *STI Review* mirrors the focus of the OECD Directorate for Science, Technology and Industry (DSTI), its Biotechnology Unit and the Working Party on Biotechnology (WPB), and the breadth of their work. There has been a significant evolution in the pattern of OECD's engagement with biotechnology. During the 1980s, policy studies addressing various genetic questions about biotechnology or recombinant DNA remained the preoccupation of the Committee for Scientific and Technological Policy (CSTP); the work on safety was delegated to its Group of National Experts on Safety in Biotechnology (GNE). Scientific success led, in the 1990s, to biotechnology's application in a growing number of sectors – the responsibility in policy terms of Agriculture, Health, Environment and other Ministries – and to a similar expansion and multiplication of work at the OECD. In 1994 the Committee for Scientific and Technological Policy consolidated its interests into a Working Party on Biotechnology (WPB). Work under other Committees and in the corresponding OECD directorates developed in

function of their diverse specific responsibilities, particularly under the Environmental Policy Committee, its subsidiary bodies, and the Environment Directorate, pursuing, amongst other issues, the harmonization of regulatory oversight in biotechnology through a new expert group, created in 1994. Transparency and co-ordination among the various OECD activities is ensured by the creation (also in 1994) of an Internal Co-ordination Group on Biotechnology (ICGB). The present *Review,* while reflecting this breadth, addresses it from the DSTI perspective.

It is thus no coincidence that nearly all the chosen authors have played, or are still playing, a distinguished role in DSTI's biotechnology work, as experts, co-authors of published reports and initiators of major projects; in one case as chairman of the GNE and in another as an OECD staff member responsible for an important activity. All have helped to shape the Organisation's work and outlook, and through it have in many ways informed and influenced government thinking and policies, at least indirectly.

The sequence of the seven articles reflects the evolution of biotechnology applications over the last 20 years, not current success stories, nor long-term priorities. The human health sector, including medical research, was the first to encounter, the first to be affected in depth by, the new biotechnology. Impacts on the agricultural and food sector (crop plants and animals) soon followed. Bioremediation and other applications in the environment were slower to develop. Only recently has the long-term importance of the environmental opportunities and needs for biotechnology been recognised as equal to, perhaps greater than, the significance attached to health and agro-food biotechnology.

Of the three main sectors, it is clear that many health and environmental applications are already, or are on the way to becoming, success stories – scientific/technological and economic. To date, agro-food applications are not.

No observer who contrasts global population growth with decreasing productivity growth rates in agriculture will question the long-term need to apply modern biotechnology to crop and forage plants, and to conversion (and storage) efficiency in the whole agro-food chain.

However, the application of modern biotechnology – and later, gene technology – to current food production in industrialised countries has run into troubles from its very beginning in the 1970s, when enzyme-based industrial sweeteners started to challenge the established interests of traditional sugar producers.

A pattern of reaction has emerged since then which has bedevilled the development of a number of agro-food biotechnologies in industrialised countries. Sectors threatened by productivity-increasing and job-cutting agro-food biotechnologies create alliances with other groups opposed to biotechnology for other reasons. The new biotechnologies, be they bovine somatotropin ("bovine growth hormone"), transgenic tomatoes or transgenic animals, have faced challenge or

delay. The arguments advanced include important questions of safety, health or ethics, but often make little mention of the original source of the opposition: the wish to protect agricultural markets and employment.

On the other hand, the companies financing the development of agro-food biotechnologies may over-emphasize the regulatory hurdles that have been used as instruments of delay. The root cause of their problems lies in the absence of a genuine demand pull for new biotechnology-derived foodstuffs. In industrialised countries, general food markets have long been saturated. One of the few ways to open up new, specialised food markets is to associate new foods with the convenience, nutritional, health or ecological demands of the public. Biotechnology can help to meet these needs, but is not the only, and often not the most readily accepted, tool for developing new foods to meet these public demands and hopes.

Food biotechnology is indeed contributing to food quality and safety, and to cost reduction. However, the lack of evident demand is a fundamental economic difference, distinguishing it from health, and environmental, biotechnologies. For the same reason, this issue of the *STI Review* places relatively less emphasis on food biotechnology.

Health and environmental biotechnologies respond to articulate, major social pressures and economic demands, which will grow quickly over time. It is sufficient to glance through the first and the fifth articles – "Biotechnology and the New Revolution in Health Care and Pharmaceuticals" and "Environmental Biotechnology for the Millennium" – to appreciate humanity's almost unlimited health and environmental needs and demands on the one hand, and the enormous potential to respond to these needs, which the versatility and rapid progress of biotechnology will deliver, on the other. For food, and particularly food crops, the current saturation will give way to major new needs and demands over the medium and long term, triggered by environmental degradation and climate change in conjunction with population growth.

This brings us back to the underlying "coherent" theme of this *Review*: the "pervasiveness" of biotechnology. The term "pervasiveness" was coined by economists (Christopher Freeman and others) in the 1970s and 1980s, studying the impact of new technologies on the economy and seeking to determine the conditions under which a cluster of radically new technologies, a "technological paradigm", would lead to far-reaching changes throughout the economic system and society. Such changes would profoundly modify education and training, employment and skill profiles, capital stock, legal systems, even language, basic social notions and organisation, and would thus require at least a generation to be completed. The two main cases studied were the replacement of steam power by electricity in the 19th century and the informatics and telecommunications revolution of the 20th century. Indeed, these technologies radically transformed our

economies and societies, creating permanent changes, assimilated over decades. Would the "biological revolution", its technological offspring and their applications, be of comparable impact? Economists were uncertain, unconvinced.

Twenty, even ten, years ago, it was still difficult to assess the range of possible, or probable, applications. There is a major difference between the diffusion process for a single, even radically different product – and no one doubted that biotechnology would generate a few of these – and the diffusion process of a new, generic technology, with numerous applications in a vast number of economic sectors, leading to a new technological paradigm and a new technological consensus for a generation of scientists, engineers, industrialists and consumers.

To create a new generic technology and to achieve the major economic and social changes referred to, the experience of the electrical and informatics revolutions indicates that five conditions should be fulfilled:

- a new range of technically improved products and processes;
- cost reductions for many of these;
- social and political acceptability;
- environmental acceptability;
- pervasive effects throughout the economic system.

Information technology satisfied all five criteria, which helps to explain the depth of economic and social transformation that it has brought about in one generation. The case of biotechnology was less clear-cut. While it was recognised that biotechnology would generate a large number of improved products and processes, some at reduced cost, acceptability was seen to be assured for some applications of biotechnology *(e.g.* health), but not for others *(e.g.* agro-food).

The key criterion of the five just mentioned is "pervasiveness". It is the pervasive effects of a new technology throughout the economic system which confirms the emergence of a new "technological paradigm". It is also the criterion where opinions considering the future of biotechnology were particularly hesitant and divergent.

Experts and observers of the new technology from the early beginning discussed its "pervasiveness", sometimes without using this term, except for a group of specialised economists. How far would the new technology go? If it was a "revolution", as some scientists had claimed, did it at least have an industrial future? Would it ever lead to marketable products and processes?

The debate on "pervasiveness" can, roughly speaking, be separated into three periods. The first lasted through the 1970s into the early 1980s, the second from the mid-1980s to the mid-1990s. During the first period, modern biotechnology, particularly DNA recombination, was seen as a scientific revolution with

important research consequences and with the potential to lead to a number of successful new products, primarily in the health sector. Some commentators were circumspect: biotechnology was just one more device in the tool-kit of the chemical industry. Others, more dramatically, saw the beginning of a new period where the genetic engineers' ability allegedly to "play God" would have unforeseen consequences, while again others, more enthusiastically, proclaimed biotechnology as the "greatest revolution since the invention of agriculture".

Such claims were widely derided. Biotech "hype", as it was called, even stimulated its own social science research: a young French sociologist of science submitted a paper to the OECD that analysed biotech hype and the safety discussions regarding rDNA technology as ideologies which disguised the subjective economic interests of the proponents of biotechnology. In its first publication on the subject, *Biotechnology: International Trends and Perspectives,* in 1982, the OECD defended the main position mentioned above: biotechnology was a revolution indeed, but it had still a very long way to go. The longest chapter of the book was that on "constraints".

A few years later, the hypothesis that biotechnology could one day become a new technological "paradigm" started to be discussed as a serious proposition, amongst others in OECD's *Biotechnology: Economic and Wider Impacts* (1989, but completed earlier). Not only had the number of new biotechnology products reaching the market increased, but they were accompanied by a few surprises. The emergence of rDNA "fingerprinting" as a major tool of forensic science impressed observers; such an application had been completely unforeseen. Equally impressive was the newly achieved industrial mass production of interferons, and other high-value molecules, by genetically modified micro-organisms. This had become a reality much earlier than cautious forecasters had predicted a few years before. It was now recognised that biotechnology was more "pervasive" than more narrowly focused technologies, as it had already found applications in agriculture and forestry, in chemicals, drugs and food, and in tertiary industries (health care, research) as well. However, biotechnology was still not seen as comparable to information technology, which had been able to penetrate virtually all products and processes of human activity. Biotechnology, in contrast, was said to be limited by the fact that it operated through living organisms or parts thereof, which seemed to restrict its field of activity to materials that could be biologically manipulated.

Since approximately 1994-95, the general outlook on the future of biotechnology and hence its "pervasiveness", has again started to change.

No longer is there any doubt that biotechnology and the increasing number of new products and processes it had brought or was about to bring to the market were heralding economic and social change in many sectors.

In OECD countries, more and more Ministries (and consequently, inside the OECD, more and more Directorates) were getting involved in biotechnology, taking responsibility for sector-specific applications or particular policy issues.

Biotechnology regulations, the source of so much argument in the 1980s, were in place in most countries (or could be applied under existing statutes), apparently without causing unsolvable problems for industry. The issue to be addressed now is harmonization. The focus of international interest – and possibly conflict – was shifting to intellectual property protection in biotechnology and to trade issues. What better proof that biotechnology has finally moved from the realm of conjectures to the real world, where exports and money are at stake?

In the domain of human health, the revolution triggered by the new knowledge and technology, and predicted by a few experts 20 years ago, is in full swing. It is raising economic, social and ethical questions of a more far-reaching nature than any previous individual discovery or group of discoveries in this sector.

It is clear that biotechnology will have a more profound effect on our "quality of life", on how long and how well we live, than any other technology, including information technologies. However, whereas it has been possible to demonstrate the enormous impacts of the "informatics revolution" by macroeconomic yardsticks, such as job creation and productivity growth, comparable statistics barely exist for technology impacts on health. "Quality of life" cannot be measured except by indirect means, and some of the data that do exist, *e.g.* on increasing life-expectancy, are ambiguous and can be seen as "good" or "bad", according to the perspective one takes.

If the second important sector of application, the agro-food sector, has seen, and will continue to see, delays at least in the short term, the third sector, the environment, which was barely mentioned 20 years ago, is becoming a major market for biotechnology. Increasingly severe pollution problems, and the growing sensitivity of the public all over the world, are guarantees that demand for biotechnology from this sector will not only remain, but will grow fast for as long as populations increase, economic development continues and material standards of living rise.

Air, soil and water pollution is ubiquitous and concerns all of humanity. With an appropriate economic and policy framework, the ultimate market for bioremediation could be, if not unlimited, then very large indeed.

Water appears to be the most critical constraint, and one that could decide between peace and war in more than one region of the world. Twenty years ago, water was mentioned in the context of biotechnology only because it was the oldest and, in tonnage, the largest sector of application of "traditional" biotechnology. Already in the 19th century, the countries of the northern hemisphere intro-

duced biological cleaning of municipal waste water. Ironically, however, no one saw water emerging in the 21st century as one of the priority targets not for one, but for many different types of biotechnology, as is amply demonstrated in Aceves' article on "Biotechnology and Water Conservation". Two conclusions can be drawn from this. First, as alluded to already, there is an important element of surprise in all technological revolutions – needs and markets emerge, and solutions spring up where least expected. Second, comparisons between different technological revolutions in history can become very misleading. The discussions on the "pervasiveness" of biotechnology that took place during the 1980s (the so-called "second period"), including those in the OECD, were literally obsessed with the precedent of the information revolution, which provided all the benchmarks, the measures, the targets.

In comparison, biotechnology was seen as more limited than information technologies, as it was "restricted to materials that could be biologically manipulated" (see above). Water is not an obvious example of such a "material", yet biotechnology can both clean water and prevent water pollution, can conserve water and fight desertification, and can save water by improving the technologies of the main water users, particularly in agriculture. "Pervasiveness" indeed; but not such as could be easily derived from comparisons with information technologies.

Two other fields of application within the environment sector are likely to grow in importance: one in the medium term; the other, more unexpectedly, in the very long term. Both are included in the article on "Environmental Biotechnology for the Millennium". As environmental policies shift their main goal from pollution clean-up to pollution prevention, technologies that help industry not to pollute receive increasing attention and support. Attention focuses on the replacement of waste-creating industrial production processes by clean or cleaner ones, often derived from biotechnology (*e.g.* the replacement of traditional catalysts by bio-catalysts). Sectors that will be modified by such clean technologies include food, animal feed, chemicals, pulp and paper, textiles and leather, energy. Also included are metals and minerals – not originally thought of as industries where biotechnology would find easy access. Such industries represent a very large part of the manufacturing sectors of nearly all countries, which will greatly add to the "pervasiveness" of biotechnology.

Finally, global climate change, or rather, its prevention or mitigation, could become a fascinating long-term goal for biotechnology. This would include both the reduction of CO_2 and methane emissions, which contribute to climate change, by new agricultural approaches; and the increased absorption of such emissions, *e.g.* by marine micro-organisms or trees. The potential of biotechnology to address climate change has been demonstrated, but it will take time before national and international authorities understand and admit it, which is one of the

typical features of all technological revolutions. New trees, not new cars? It looks too simple!

So, is biotechnology a new "paradigm"? Yes, if the compulsory reference to the yardsticks of earlier technological revolutions, particularly electricity and informatics, is finally dropped. Biotechnology is *sui generis* because it deals with life – and life ultimately escapes the statistical constraints necessary for objective comparisons and for testing what is essentially a macroeconomic theory.

This issue of the *Review* amply testifies to the impressive and steadily growing "pervasiveness" of biotechnology. In fact, so "pervasive" and varied has biotechnology become that this could well be the last time that the OECD reviews the entire field as if it were a unity.

It is worthwhile to summarise, and reflect on, the reasons that have made it difficult to get the propositions of "pervasiveness" and "technological revolution" accepted for biotechnology.

One reason, already mentioned, is the varied, disparate and apparently unlinked character of many applications. Breast cancer, improved cereal strains and global climate change? The link is not obvious at first sight, and would ultimately have to be explained scientifically in terms of the common genetic code, common evolutionary origins – concepts not readily apparent. In contrast, the coherence and centrality of the information concept have made it easy to demonstrate the validity of the "technological paradigm" theory with examples from the information revolution. As information pervades every type of human activity, an information revolution must also modify every type of human activity.

A second reason lies in the, apparently unchangeable, bias of many life scientists who for at least 30 years have persistently underestimated the speed of scientific development of their own disciplines, as well as the possibilities of application. Even in this *Review,* several references can be found which indicate that in this or that sector or discipline "progress has been faster" than expected. This is not the place to speculate on why biologists have been continuously overcautious when forecasting progress in their own fields, whereas physicists have usually been over-optimistic. Suffice it to say that biologists, particularly in the long-established disciplines of biochemistry, microbiology and genetics, were reluctant to acknowledge the ultimate scope of the molecular revolution and to accept the broad concept of a new, "biotechnological paradigm".

A third reason, finally, lies in the relationship of biotechnology to other technologies, particularly those based on chemistry, which is not directly comparable to the way new technologies displaced older ones during earlier technological revolutions.

Electricity drove steampower out of its major applications, even if it took a long time. The new information technologies based on electronics and computers

completely eliminated, when available, the technologies pre-dating the invention of the transistor. The relationship was one of radical substitution.

Biotechnology, in contrast, invades, complements, competes with older technologies, and in some instances, even stimulates other disciplines, particularly chemistry, to improve. It is true that in a growing number of cases, particularly in the health field, it will also replace older technologies and find solutions that no other technology could provide. Nevertheless, synergy has so far been more typical of biotechnology than radical substitution, which has made it more difficult to see the revolutionary or "paradigmatic" component of the technology. Again, biotechnology is *sui generis*...

Salomon Wald

BIOTECHNOLOGY AND THE NEW REVOLUTION IN HEALTH CARE AND PHARMACEUTICALS

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I. INTRODUCTION AND PUBLIC HEALTH: TOWARD THE 21st CENTURY

In the past few years there has been sudden and extraordinary progress in biomedical research, due primarily to the application of new biotechnology, *i.e.* recombinant DNA techniques and hybridoma technology, to human diseases. It now appears evident that the current and potential applications of the new biotechnologies in medicine amount to essentially a new approach to drug discovery/design, production and delivery. The new advances come with the promise to address and resolve problems on frontiers of medicine that have resisted traditional methods and interventions. The genetic defects that predispose for various forms of heart disease, breast and colon cancer, diabetes and arthritis have been identified. The genes responsible for cystic fibrosis, Duchenne muscular dystrophy, neurofibromatosis, and fragile X-linked mental retardation have been isolated. In addition, the knowledge which will result from the international research efforts to map and sequence the human genome will greatly enhance the ability to predict genetic diseases and genetic components of common diseases. Many kinds of human diseases will eventually be treated by introducing heterologous genetic information into defective or damaged tissues through gene therapy. This is reflected in the types of disorders for which gene therapy has already been attempted. Many of the recent clinical studies using the new genetic approaches involve single gene defects, neoplastic diseases and infectious diseases, such as acquired immunodeficiency syndrome (AIDS). Furthermore, to prevent human disease, biotechnology is being used successfully to develop new vaccines, such as live viral vaccines and naked DNA vaccines.

Another consequence of the new biotechnological approach is that drugs can now be discovered, developed and produced more efficiently and rapidly. Some of the proteins under development have no precedent in nature. They are made up of a combination of specific domains extracted from different proteins to produce molecules capable of novel functions. Furthermore, combinatorial chemistry, a new method to simultaneously create a large array of biologically and chemically derived peptide libraries and then test thousands of related compounds for various kinds of biological activity has the potential to fast-forward the process of drug discovery. The number of new biotechnology medicines entering

clinical trials each year is increasing at a double-digit rate, and as these products enter the commercialisation phase, gaining the appropriate regulatory approvals, as well as securing the appropriate patent rights, is becoming perhaps the limiting factor in new product development.

Thus, the rapid progress in the field, while impossible to predict in detail, raises several policy implications for industry and government. A major concern for governments is the rapid increase in health-care costs. Today, governments are grappling with limited resources and the need to assess and set research and health-care priorities, while the pharmaceutical industry has come under unprecedented pressure to prove that its new products are not only better than the existing ones, but also cost-effective and competitive.

The changing demographics of OECD countries where populations are becoming increasingly elderly, pose particular social and economic challenges. Over the coming decades there will be more old people and fewer people of working age who can provide for the elderly. Governments will have to make difficult choices in allocating funds and in setting priorities. Furthermore, most of the ailments of the aged are chronic degenerative diseases to which there are presently no therapeutic solutions. This means that the majority of these diseases can be treated only symptomatically; that is, at best, the symptoms of the disease can be alleviated, but the cause cannot be treated. The development of causative therapeutic solutions is often complex, lengthy and resource-intensive, since the mechanism of the disease must first be determined. Thus, the drugs and technologies that result from this knowledge-intensive research will invariably be expensive. In the past, new drugs and technologies were welcomed and their adoption was rarely questioned. Today, this is no longer the case. Therefore, there is a growing need to develop adequate tools for health technology assessments, and in particular, to measure the outcome of interventions in terms of "quality of life". In this increasingly uncertain environment it is no surprise that the international biopharmaceutical industry has been driven to fundamental restructuring where partnerships and alliances play an increasingly important role in sharing the risks of the development of new cost-effective solutions to increasingly complex medical challenges.

The task of reviewing, in a limited report, the coming changes in health care and in the biopharmaceutical industry, as briefly announced above, poses difficult choices. This article has been designed to provide the layperson with a notion of the science and the technology behind a number of important new biotechnological advances and policy debates, while addressing whenever possible the international issues arising from the adoption and diffusion of the new technologies.

II. CURRENT HEALTH NEEDS AND ISSUES

Health care has generally improved over the past 40 years. In 1900, infectious disease was the leading cause of mortality in the United States and many European countries, accounting for at least 37 per cent of deaths. By 1993, the frequency of deaths by infectious diseases in the established market economies had dropped to 1.5 per cent, with corresponding improvements in life expectancy. Today, three out of every four deaths in the developed world are due to non-communicable diseases. Diseases of the circulatory system – heart attacks and strokes – are the largest single cause of death in developed countries since they account for about 46.7 per cent of total deaths, while malignant neoplasms account for 21.6 per cent of deaths. By contrast, one in two deaths in the developing world (or 41.5 per cent of total deaths) is still caused by communicable diseases, including maternal and perinatal causes.

According to the *World Health Report* of 1995 (WHO, 1995), globally about 51 million people of all ages died in 1993. Some 39 million deaths took place in the developing world and about 12 million in the developed. As far as specific causes are concerned, of the 20 million deaths due to communicable disease, more than 16 million, or about 80 per cent were due to infectious and parasitic diseases. Tuberculosis killed about 3 million people, hepatitis-B possibly 1 million and malaria around 2 million. Since the early 1980s, one of the most elusive illnesses that has affected humankind is AIDS, or human immunodeficiency virus (HIV), infection. The World Health Organisation (WHO) estimates that in 1994 HIV prevalence among adults world-wide was over 13 million. By the year 2000 the cumulative total of HIV infections could reach 30-40 million.

Associated with HIV, multi-drug resistant tuberculosis could also become one of the leading causes of adult deaths in some countries. HIV damages the immune system and accelerates the speed at which tuberculosis progresses into a life-threatening disease. It is estimated that tuberculosis killed some 3 million people in 1993, representing more than 5 per cent of deaths globally. There were an estimated 8.8 million new cases in 1995. This corresponds, according to the WHO, to 52 000 deaths from the disease per week, or over 7 000 each day. The bacille Calmette-Guérin (BCG) vaccine, developed in 1921 and still the only vaccine against the disease, is effective in protecting infants from severe forms of childhood tuberculosis, but provides little protection for adolescents and adults. The consequences are that in sub-Saharan Africa at least 3.8 million people are infected with both tuberculosis and HIV, while Asia, with 1.1 billion carriers of tuberculosis, faces a similar potentially devastating explosion of AIDS-related tuberculosis.

In addition to these communicable diseases, congenital and hereditary abnormalities are still a major cause of human illness and death in the first year of

life. According to the WHO, hereditary diseases are a growing public health concern: 25-60 per 1 000 liveborn infants are estimated to have congenital abnormalities. Without any doubt the scale of the problem is significant and justifies the increased emphasis on genetic research.

As a final point, the increasing number of elderly people is, or will become, a major concern in most societies. According to the WHO, the world's population has been growing at an annual rate of 1.7 per cent during the 1990-95 period, but the population over 65 years is increasing at a much faster pace, by some 2.7 per cent annually. The implications of this growth are cause of great concern since most of the ailments of the elderly are chronic degenerative diseases for which there is little cure, such as arthritis, dementia, cancer, etc. Furthermore, does this extended life mean better health, or simply more years of sickness and dependence on health and social services? Therefore, there is a growing need of developing adequate tools for health technology assessments, and in particular, to measure the outcome of interventions in terms of "quality of life".

Thus, despite the great medical achievements of the first half of this century, the battle to improve health has yet to confront the difficult task of the diagnosis and treatment of genetic, cardiovascular, neurological, viral and autoimmune diseases, cancer, the scourge of HIV and multi-drug-resistant tuberculosis and new emerging infections such as the Ebola virus and bovine spongiform encephalopathy (BSE). This is the challenge facing biomedical researchers in academic settings and industry, clinicians and public health professionals. The confident prediction is that through new biotechnology more efficient vaccines against bacterial and parasitic diseases are likely to be developed in the near future and that by the end of this century new diagnostics and genetic therapy may be ready for application in the prevention and treatment of many of the current diseases.

III. PHARMACEUTICAL BIOTECHNOLOGY AND NEW APPROACHES TO DRUG DISCOVERY

Biotechnology is an integral part of a new approach to medicine and to drug discovery, production and delivery, which primarily uses two methods developed in the 1970s: recombinant DNA technology and monoclonal antibody technology.

Recombinant DNA technology, also called genetic engineering, has been widely used to produce natural proteins in large quantities since the successful development in 1978 of the first genetically engineered bacteria able to produce insulin. Since then, and in particular during the past decade, the biotechnology industry has produced many new drugs through the use of genetic engineering (see Table 1). For example, tissue plasminogen activator, a natural enzyme that

Table 1. Examples of approved and advanced biotechnology drugs/vaccines for medical use

Application	Product
Autoimmune diseases	
Multiple sclerosis	IFN-β
Rheumatoid arthritis	TNF-α antibody
Blood deficiencies	
Anemia	Erythropoietin
Blood substitute	Hemoglobin
Chemo-induced	G-CSF
Hemophilia	Factor VIII
Cancer	
Bone marrow transplant	GM-CSF
Leukemia	IFN-α
T-cell lymphoma	IL-2 fusion toxin
Melanoma	IL-2/ melanoma vaccine
Renal cancer	IL-2/ IFN-γ
Cardiovascular diseases	
Myocardial infarction	tPA
Angina/restenosis	GP-IIb/IIIa antibody
Genetic diseases	
Cystic fibrosis	DNase
Diabetes	Human insulin
Gaucher's disease	Glucocerebrosidase
Growth deficiency	hGH
Infectious agents	
Hepatitis-B virus	IFN-α/ subunit vaccine
HIV	IFN-α/ IL-2
Papilloma virus	IFN-α
Bordatella pertussis	Acellular vaccine
Inflammatory disorders	
Allergy	IgE antibody
Graft-versus-host-disease	tac antibody
Septic shock	BPI
Nervous system disorders	
Amyotrophic lateral sclerosis	IGF-1
Trauma	PEG-SOD
Tissue damage	
Wound healing	TGFβ/PDGF

Source: Adapted from Kirston Koths (1995), Recombinant Proteins for Medical Use: The Attractions and Challenges", *Current Opinion in Biotechnology*, No. 6, pp. 681-687.

dissolves blood clots, is currently used to help prevent life-threatening damage to the heart; genetically engineered lymphokines and growth factors are used in the treatment of cancer and alpha-interferon is the only treatment for hairy cell leukemia. Similarly, the genetically engineered interleukin-2 and granulocyte colony stimulating factors are used to induce and potentiate the body's defenses against cancer and AIDS. Furthermore, recombinant DNA technology has been exploited

for the development of vaccines, such as the now widely used hepatitis-B sub-unit vaccine. Specific genes from the AIDS virus have also been isolated with the hope of producing *in vitro* fragments of virus protein for experimental use as an AIDS vaccine.

Monoclonal antibody technology allows scientists to produce antibodies by fusing an antibody-producing white blood cell to a cancer cell. The result is a cell which can reproduce indefinitely and has the ability to make antibodies in large amounts. Because of their exquisite specificity of action and target recognition, antibodies produced in this way have been successfully used to develop diagnostic tests for the detection of hepatitis, venereal disease, bacterial infections and, most recently, HIV. In addition, monoclonal antibodies that carry minute amounts of radioactive material can be used in combination with computerised technology to locate and study specific diseased tissues *in vivo*.

A relatively new technology which may replace monoclonal antibodies for *in vivo* diagnostics and therapeutics is based on the design of peptides with specific binding capabilities. In fact, it has been argued that peptides might prove to be the reagents of choice for the future for a variety of reasons including their small size which facilitates *in vitro* synthesis and delivery. Recent advances in computer-aided drug design include attempts to deduce the structure of a target molecule from, *e.g.* the three-dimensional shape and surface properties of its ligands. The most difficult aspect of this technology is the ability to predict biological activity, *e.g.* whether a designed molecule will bind a target molecule, and will act as a potent inhibitor, or will bind poorly or not at all. However, this may change soon due to the progress in yet another new technology – "combinatorial chemistry" – that will facilitate the testing of thousands of compounds at the same time.

Combinatorial chemistry was born about a decade ago when researchers at a start-up biotechnology company in San Diego (Affymax) first devised this technique as a way to generate libraries of simple-protein-like molecules. Conventional product discovery and development involves numerous, time-consuming, expensive, and at times inefficient, cycles of testing of individual product candidates. By contrast, combinatorial chemistry has the potential to replace this traditional approach with a method that facilitates parallel synthesis and evaluation of thousands of compounds. Conceptually, combinatorial chemistry employs the principle of natural selection that has been used over the past century to study and explain a wide range of biological phenomena. The molecules to be screened are viewed as analogous to "variants or mutants" occurring in nature. The parallel evaluation of the diverse sets of molecules in the library is considered as a sort of artificial biological selection that leads either to the emergence of an acceptable product or to the identification of a compound for second-generation refinement. As in nature, ideally the ultimate screen would be in the form of a continuous process of generating and evaluating molecular diversity. Thus, the new technol-

ogy represents an exciting opportunity that bridges knowledge and skills not only in chemistry and biology, but also in materials science, instrumentation, applied mathematics, statistics and computer sciences.

IV. THE HUMAN GENOME PROJECT: DIAGNOSIS AND PREVENTION OF DISEASE – THE WORLD OF GENOMICS

In less than seven years since its inception in 1989, the Human Genome Project, a major international programme to map and sequence the human genome has seen unexpected progress. Many of the project's goals, which seemed optimistic when they were first proposed, are now considered possible. The initial effort to develop a genetic map of the human genome, *i.e.* a map where specific molecular markers are associated with the inheritance of genes, has already proven successful. A 2.5 centimorgan genetic map (*i.e.* a map in which landmarks are spread on average at 2.5 centimorgans) will soon be completed. The physical maps of the Y or male chromosome and of chromosome 21, which houses genes involved in Down's syndrome, Alzheimer's and other neurological diseases, are now completed and maps of chromosomes 19 and 16 are well advanced. Each map is an actual representation of the chromosome, consisting of DNA clones pieced together in the correct order, tagged by long sequences of DNA (generally occuring only once in the whole genome) that can be used as common reference points. This sudden progress is due primarily to the development of new cloning techniques such as yeast artifical chromosomes (YACs), which make use of modified chromosomes into which very long stretches of DNA can be inserted and amplified. As a consequence of this new achievement, large-scale physical mapping is now moving ahead faster than predicted and may in fact be completed for all human chromosomes within the next three years. Meanwhile, new strategies promise to significantly speed up sequencing.

To date the Human Genome Database contains about 1 109 genes, associated with 1 223 human genetic disorders. Furthermore, the initial ordering of DNA *loci* with markers along the chromosome constitutes a map with many virtues, as it is necessary for locating new disease genes. For example, although glukokinase has long been considered a candidate gene for diabetes susceptibility, it was not until recently, with the use of these markers, that the appropriate studies linking the disease phenotype with a specific gene were made possible. Once the chromosomal location of a disease-producing gene has been determined, fine structure genetic mapping can narrow down the region to be searched. In the past few years the genes responsible for cystic fibrosis, Duchenne muscular dystrophy, neurofibromatosis, and fragile X-linked mental retardation have been isolated. In addition, the genetic defects that predispose for

various forms of heart disease, breast and colon cancer, diabetes and arthritis have also been identified. At the same time, the growth of powerful computerised databases and the development of bioinformatics are bringing further insights, providing the necessary link among the data developed by the many international laboratories involved in this endeavour. The Genome Data Base, developed by the John Hopkins University in collaboration with the Howard Hughes Medical Institute, integrates various kinds of mapping and sequencing data, as well as the constantly evolving genetic linkage map. The Paris-based Centre d'Étude du Polymorphisme Humain organises data from laboratories around the world to develop a series of consensus maps for each chromosome. Another international body, the Human Genome Organisation, is now co-ordinating the efforts of 42 nations.

V. FUTURE APPLICATIONS OF HUMAN GENOME DATA: TESTING FOR GENETIC DISEASE AND ITS IMPLICATIONS

Discussions on genetic testing and population screening have taken place for many years. Indeed, the ability to predict *in utero* or at an early age the potential risk for a genetic disease already exists for several situations. Sickle cell disease, hemophilias and Huntington's disease have become the prototypical examples. In general, carrier detection is limited to families with a case history. Only in exceptional circumstances, such as with β-thalassemia, has general population screening been carried out. However, this may soon change since the information obtained from human genome mapping may be applied to human genetic epidemiology, *i.e.* for the measurement of the incidence and prevalence of a broad array of genetic disorders and for the planning of genetic health-care resources. The Human Genome Diversity Project was proposed in 1991, when a group of human geneticists and molecular biologists suggested investigating the variations occurring in the human genome by studying samples collected from populations that are representative of all the world's people, including populations that are anthropologically unique. The information generated by this approach should throw light on questions of interest to geneticists, anthropologists, historians and, ultimately, to the populations themselves; although critical reviewers (including UNESCO's International Bioethics Committee) have emphasized the need for ethical considerations in such a project – especially regarding "informed consent".

However, it is at the level of the individual that genome information will most likely enable a finer understanding of diseases. This understanding will be used in the future to "tailor" treatments to the needs of the individual patient and to

facilitate the development of preventative therapies, and ultimately of gene therapy, *i.e.* the substitution of a normal gene for a malfunctioning one.

This new approach to the identification of disease-causing genes has been called "genomics". Genomics is a direct product of the Human Genome Project, and the increasing awareness of the importance of this approach is illustrated by the number and size of the pharmaceutical companies that are becoming involved in genome research.

There is little doubt that the data obtained with the human genome project will be useful in the preparation of a genetic profile of health needs and in determining health-care priorities taking into account cost effectiveness and cost benefit. However, genetic testing also poses some serious ethical and philosophical issues. First, the new science of genomics calls into question many classical notions of disease. One could take what is at times called a "genetic nihilist point of view" and argue that all diseases are genetic. However, this approach carries us back to the notion of an organism as a "closed system", and to the "nature *vs.* nurture" type of argument. A more reasoned approach would be to view the etiology of disease through a multi-step causal pathway where the knowledge of the genetic component will help identify and target therapeutic treatments more precisely.

Furthermore, the new genomic data may shed some immediate light on the etiology of single-gene diseases, but there are still major issues that scientists as well as health services will need to address. More than 4 000 genetic diseases are known today, for most of which there is no real cure. It is reasonable to predict that the genes for these diseases will soon be sequenced and that this knowledge will speed up and help diagnosis. However, the existing medical approaches for these diseases are only of a palliative nature and are often limited to preventive measures at the embryonic stage or before marriage and/or treatments to alleviate symptoms of congenital impairment.

Furthermore, a recent study on a cross-cultural perspective on genetic counselling in 19 nations has shown that most health-care centres have difficulty in meeting the existing demand for services. While genetic testing has become increasingly available, genetic counselling and health-care have not kept pace. The ability to predict diseases and understand their pathogenesis has proceeded at a faster pace than the development of educational programmes and therapies. This is particularly exemplified by the dilemma posed by recent advances in understanding of genetic predisposition to breast cancer. In families with a high incidence of breast and ovarian cancer, 75 per cent of the affected members carried mutations in a gene on chromosome 17, called BRCA1. Thus, mutations in this gene have been associated with an estimated lifelong risk of 85 per cent for breast cancer and 50 per cent for ovarian cancer (Szabo and Kling, 1995). What would this mean if a test for BRCA1 were to be offered? Given the heterogeneity of mutations reported until now, the technical challenges of conducting a survey

for all possible mutations is quite daunting. However, even more daunting is the decision about the appropriate medical care for women with these mutations. With the emergence of somatic gene therapy, medical intervention will become an option. However, measures to reduce the burden of genetic diseases may, in most cases, require primarily programmes of education of the public and adequate training of health-care personnel.

As a final point, knowledge of the genetic make-up of an individual, including the likelihood of an individual's predisposition for a specific disease opens the door to both preventive strategies and the unwelcome possibility of genetic discrimination. The issue of the possible misuse of genetic information has been the subject of extensive debate since the beginning of the Human Genome Project. Individuals might be compelled to provide genetic information in order to access health care, and this information on genetic health risk assessment may also include the extended family. Many countries have already debated the need for legislation restricting the access of insurance companies or employers to genetic information. As a reaction to these positions, the insurance industry has drawn up a code of practice on the use of genetic information for its members. However, it has been recognised that the standard personal history is already a rich source of genetic information. Therefore, policies intended to protect genetic privacy will need to address the privacy of health-related knowledge in general. In May 1991, the joint NIH-DOE USA Working Group on the Ethical, Legal and Social Implications (ELSI) of Human Genome Research formed the Task Force on Genetic Information and Insurance to develop recommendations to prevent the negative impact of genetic information on access to insurance. One of the main points of the recommendations is the inclusion of genetic services and treatments within basic health services.

VI. THE NEW VACCINES

In recent years, adult immunisation has not received the same priority as immunisation of children. However, a significant number of deaths from preventable diseases occur predominantly in adults. To cite some numbers, between 50 000 to 70 000 adults die each year from pneumococcal infection, influenza or hepatitis-B, as compared with about 1 000 children who die from diseases targeted by childhood immunisation (Centers for Disease Control and Prevention, United States). Furthermore, several of the vaccines available for children are less efficient in adults. For example the efficiency of the pneumococcal polysaccharide vaccine has been found to decline dramatically with age (93 per cent for recipents less than 55 years old, to 46 per cent for those 85 years old). The resurgence of tuberculosis in recent years has also uncovered the controver-

sial efficacy in adults of the available bacille Calmette-Guérin (BCG) vaccine. Furthermore, immunity to many diseases is serotype specific, thus limiting the use of many of the available vaccines. For example, pneumococcal disease is serotype specific, and the available vaccine contains only 23 of the more than 80 pneumococcal serotypes. Therefore, even full implementation of the currently available vaccine would prevent only one-third of the cases of pneumococcal invasive disease. New vaccines are also needed for rotavirus diarrhoea, acute viral respiratory infections and pneumococcal meningitis. Improved vaccines are needed for tuberculosis, cholera, typhoid, measles, group C meningococcal meningitis, polio and Japanese encephalitis. Furthermore, a better combination of vaccines could reduce doses and improve vaccine stability. Hence, the urgent need for the production of new, and relatively low cost, vaccines and for a new aggressive policy of adult immunisation. A rational approach to vaccine design may come with the use of live viral and naked DNA vaccines which hold the promise for better immunisation and prevention. In addition, new vaccine methods and immunotherapies could also be applied to diseases such as cancer and AIDS, which affect predominantly the adult population, as will be described below.

Naked DNA vaccines

In the past few years, direct injections of naked DNA have been used successfully for the transfer of genes into several animal model systems. Since the original report by Wolff and colleagues (Wolff *et al.,* 1990) demonstrating the expression of genes following the direct injection of plasmid DNA into rodent muscle, this technique has been used to develop new vaccine strategies that use DNA instead of proteins.

In current techniques of naked DNA injection, the desired gene is inserted into a plasmid and is directly injected into muscle tissues. A plasmid is a small fragment of circular DNA that can reproduce itself in bacteria, but not in other cells unless they contain the necessary molecular machinery for its transcription. Plasmids are common tools in molecular biology for cloning and amplifying genes in the laboratory; they are stable and do not cause the numerous problems associated with viral vectors, such as immune responses and concerns about safety.

The advantage of naked DNA vaccination is the relative simplicity and stability of the preparation, and the fact that, once inside the cell, the foreign fragment of DNA will express the corresponding protein. The result is that bits of the protein will be carried by specialised molecules on the surface of the cell where they will signal that, to save the host, the cell needs to be killed, thus evoking a powerful cell-mediated immune response. The killing is then carried out by a class of white blood cells or lymphocytes, the cytotoxic T-cells. By contrast, standard vaccines act by stimulating antibodies. Antibodies are molecules that circulate in the blood

and bind to foreign molecules (antigens) like a key to a lock. Once they have caught and locked the foreign molecule, they "mark" it for destruction. Since viral infections are primarily intracellular, and antibodies can act against them only if the virus is released into the bloodstream, cell-mediated stimulation of cytotoxic T-cells, as with naked DNA injections, is a desirable property of a vaccine against a virus.

Recently, researchers (Ulmer *et al.*, 1993) have shown that this approach is effective in protecting against influenza virus. Influenza is a common disease, difficult to prevent since influenza viruses can acquire many mutations that alter their external surface or "envelope" proteins, against which vaccines are usually raised. These mutations are an important reason why current vaccines fail to protect from reinfection with different strains of influenza in following years. By contrast, the naked DNA vaccine of Ulmer and colleagues was produced from a core gene (nucleoprotein) of the virus that is conserved in most strains. This vaccine was shown effective in protecting mice against lethal doses of different strains of influenza virus and performs better than existing vaccines in inducing an immune response in non-human primates. The reason for this efficacy appears to be related to the cell-mediated immune response and to the fact that the "foreign injected DNA" is degraded more slowly and lasts longer than antigens from conventional vaccines. As a consequence the immune response is stronger. With this evidence at hand, researchers now believe that naked DNA injections could also be used to "boost" the immune system when a patient's protective immune response is inadequate against active viral infections. The underlying hope is that by stimulating cell-mediated immunity it may be possible to tip the balance in favour of a stronger response against an active viral infection. This approach is now being tested to create a new type of vaccine in HIV-infected patients. This vaccine would act on an already infected HIV patient to help stimulate T-cell killing of the virus. Studies are now underway in a 15-centre clinical phase 2 trial to evaluate the benefit of this approach.

Despite these optimistic prospects, various questions remain unanswered, in particular on the long-term effects of the injections: Would the DNA insert into the host genome? What effect would there be if the injected DNA expressed the foreign protein for a prolonged period of time? All these issues, nevertheless, can be easily addressed, and naked DNA transfer holds promise in the development of vaccines that protect against multiple strains of a given virus and as a treatment for chronically active viral infections.

Cancer vaccines

In 1883 William Coley reported tumour regression in a patient "vaccinated" by co-injection of tumour cells with bacterially derived products. In the past few

decades, a similar strategy has been used to treat patients with solid tumours. Cancer vaccines differ from classical viral vaccines because the vaccine is administered subsequent to, rather than before, the pathogenic insult. This "delayed" vaccination approach is dictated by the current difficulty of predicting the antigenic characteristics of most tumours. Thus, the vaccine can be produced only by using the patient's own tumour cells. The treatment consists of injections of irradiated autologous or allogeneic tumour cells, tumour lysates, or in some cases of irradiated virus-infected cells together with adjuvants such as the BCG tuberculosis vaccine (bacille Calmette-Guérin) or more recently, with cytokines. However, the ultimate goal of tumour vaccine design is the generation of antigen-specific vaccines. Recent results on the reactivation of a specific embryonic gene product (MAGE1) in 50 per cent of adult melanomas and 25 per cent of human breast tumours (BRCA1) have rekindled excitment about this approach. A specific embryonic protein reactivated in tumour cells represents a good candidate for the production of a safe adult vaccine. The embryonic gene could be inserted in recombinant vaccinia viruses and injected in adults for preventive immunisation. Currently, modified versions of vaccinia vectors with reduced risk of potential virulence are under investigation in animal models.

Most recently another approach to cancer treatment has involved the generation of tumour cells engineered to secrete various cytokines (see section on somatic gene therapy). Some of these cytokines, when produced by tumours, induce a local inflammatory response that results in the elimination of the injected tumours. Defective retroviral vectors and adenovirus-based systems are being developed for high-efficiency transfer of genes encoding cytokines.

Somatic gene therapy

Today, it is the gene itself which is being developed as a drug for therapeutic use, and recent scientific advances have made the clinical testing of somatic gene therapy a reality. Gene therapy can be defined as a therapeutic technique in which a functioning gene is inserted into the somatic cells of a patient to correct an inborn error or to provide the cell with a new function. The successful insertion of a functioning gene leads to the expression of a gene product that is intended to supplement or replace a defective gene or to treat the effects of an acquired disease such as cancer.

Current methods for gene therapy make use of directly harvested cells, cultured cell lines, genetically modified cell lines and viral vectors, among which modified retroviruses or adenoviruses. In the *ex vivo* approach, somatic cells, including blood or bone marrow cells, tissues or organ samples, are removed, cultured and exposed to viral or non-viral vectors (see the section on new challenges to drug delivery) or DNA containing the gene of interest. Following inser-

tion (by various means) of the gene into these cells, they are re-administered to the patient. In the *in vivo* approach, viral or non-viral vectors or simply "naked DNA" are directly administered to patients by various routes. A third approach involves the encapsulation of gene-modified cells and the reversible introduction of an encapsulated cell structure, often termed an "organoid", into the human body.

Several vectors or systems are used to deliver genes into cells. Retroviral vectors offer the most promising prospect for the transfer of useful gene sequences into defective tumour cells since they target only dividing cells and have the potential of long-term expression.

Clinical trials in the United States and Europe are currently evaluating the genetic treatment of various diseases (Table 2). Gene therapy appears to have already succeeded in treating the symptoms of patients with hypercholesterolaemia and several children with severe combined immunodeficiency diseases [caused by the lack of the enzyme adenosine deaminase (ADA)]. In this latter case the methodology involved the *ex vivo* expansion of bone marrow progenitor cells which were transduced *in vitro* with a retroviral vector containing a normal human enzyme and re-inserted in the patient. Other clinical trials in the United States, France and the United Kingdom are exploring the efficacy of a treatment for cystic fibrosis through the direct inhalation of the normal gene carried in a liposome mixture or in modified adenoviruses. However, one of the most significant developments in this field is the application of this methodology to gene marking in the study of the biology of cancer.

Table 2. Diseases in gene therapy trials

Cancers
Brain tumours
Breast cancer
Lymphoma
Leukemia
Renal carcinoma
Melanoma
Myeloma, multiple
Non-small cell lung cancer
Genetic diseases
Cystic fibrosis
Hypercholesterolemia
Gaucher's disease
Adenosine deaminase (ADA) deficiency
SCID
Other
HIV

Source: American Cancer Society, Centers for Disease Control and Prevention.

Although gene therapy was originally targeted toward single-gene or monogenic deficiency diseases that are recessive and relatively rare, about 75 to 80 per cent of the current clinical trials now focus on cancer. A variety of approaches are being attempted, the most promising of which is a form of immuno-therapy via the *ex vivo* expansion of the patient's malignant cells which are engineered to express a cytokine (*e.g.* interleukins or granulocyte-stimulating factors), and then re-injected in the patient to induce an immune response against the tumour.

By the end of 1994, 100 clinical protocols involving nearly 300 patients worldwide had been approved. These protocols addressed nine different monogenic deficiency diseases, including three types of severe combined immune deficiency, familial hypercholesterolemia, Gaucher's disease, alpha-1-antitrypsin deficiency, Fanconi's anaemia and cystic fibrosis.

However, despite these encouraging results, the field awaits answers to many unresolved questions. An area where enormous progress has been made, but where much more needs to be accomplished, is in developing gene delivery vectors. Virus-based vectors have been the most efficient for inserting genes into cells in the laboratory, but in clinical applications the results have in some cases been short-lived, and there have been unwanted side-effects.

In addition, the field needs to develop animal models to test the biological and clinical efficacy of the new vectors and procedures. As a result, gene therapy may take longer to reach patients than originally predicted. Furthermore, the progress of gene therapy depends upon adequate technical, financial and training resources, and demands close interaction between academics, clinicians and private-sector companies. The importance and the complexities of this interplay between the private and the public sectors were recently discussed at an OECD meeting on "Gene Delivery Systems", held in Ottawa in 1995 (OECD, 1996).

Private companies are playing an increasingly critical role in promoting the development of technology. They have raised hundreds of millions of dollars to enter the field and, for the companies involved, this translates into financial risk: they have to choose which technologies to back, knowing that the ultimately successful approaches are likely to require complex assemblies of new clinical tools and procedures. For industry, a necessary incentive is the award of exclusive protection of their innovative breakthroughs. However, gene therapy can be viewed as the product of an assembly line, and limited access to enabling technologies – such as vectors – because of time-consuming and expensive licencing processes, could lead to prohibitive commercial burdens and delay the progress of the field. Thus the need, during this early phase of discovery, for an efficient co-ordination between an academic and a pharmaceutical rationale.

VII. NEW CHALLENGES IN DRUG DELIVERY

Medical administration of engineered protein and peptide substances requires a detailed knowledge of the stability properties of the active agent and information on the potential for local degradation or metabolism of the active substance prior to and during absorption. Thus, the advent of peptide drugs, not unexpectedly, has brought new challenges in drug delivery. As a consequence, approaches to the delivery of biotechnology products include the development of careful evaluations of pharmaco-dynamic properties coupled with new methods for local and systemic delivery to prevent or minimise side-effects while optimising efficacy.

Research has recently been focused on methods for enhancing drug bioavailability, reducing toxicities, targeting specific organs and modifying drug pharmacokinetics. The challenge of addressing effective delivery of biotechnology products has lead to the development of transdermal, gastrointestinal and nasal delivery systems. Transdermal therapeutic systems consist of thin, flexible bio-erodible polymer-membranes which include a reservoir containing the drug and are applied as a small adhesive bandage. The drug permeates through the skin and into the bloodstream at a rate regulated by the membrane. Transdermal scopolamine is currently used to prevent motion sickness. Transdermal nitroglycerin serves for the prevention of angina. The "nicotine patch" delivers nicotine for 24 hours as a smoking cessation aid. Transdermal estrogen membranes are used for oestrogen replacement. Alternatively, non-erodible osmotic implants are used for subcutaneous or intraperitoneal peptide delivery of a duration of between one week and one year.

A significant challenge is the intracellular delivery of highly charged molecules, such as oligonucleotide for antisense or antigene DNA therapy and gene replacement therapy, *i.e.* for treatments designed to replace a disfunctional gene or to interfere with its function.

The first truly efficient gene-transfer vehicles were developed in 1981 and 1982 and were primarily based on retroviruses. These vectors allowed *in vitro* stable genetic modification of a large number of cells and *in vitro* complementation of genetic defects, such as for example, HPRT deficiency and adenosine deaminase. Since then, a number of other kinds of viral and non-viral techniques have been developed for gene transfer into dividing and non-dividing cells (Table 3). The new methods include vectors derived from adenoviruses, adeno-associated viruses and herpes viruses, as well as non-viral gene-transfer techniques using lipofection and electroporation. Several of these approaches are now allowing direct gene delivery *in vivo*.

Retroviral vectors are one of the best characterised viral vectors for human gene transfer. The most frequently used virus is the Moloney murine leukaemia

Table 3. **Examples of gene delivery systems and target tissues**

Delivery system	Tissues	Target cells
Viral vectors		
Retrovirus	Bone marrow	Hematopoietic stem cells
	Muscle	Myoblasts
(with partial hepatectomy)	Liver	Hepatocyte
(adeno-associated virus)	Liver	Hepatocyte
	Blood vessel	Endothelium
Adenovirus	Muscle	Myofibres
	Liver	Hepatocyte
	CNS	Neuron
	Lung	Epithelium
Adeno-associated virus	Liver	Hepatocyte
Herpes simplex virus-1	CNS	Neuron
Physical methods		
Receptor-mediated	Liver	Hepatocyte
Direct injection	Muscle	Myofibres
Lipofection	Lung	Epithelium
	Blood vessel	Endothelium

virus (MoMuLV); this virus replicates both in mouse and human cells. This dual tropism is particularly useful since it allows testing of the constructs in mouse cells. In order to prevent unwanted *in vivo* viral replication, the viral structure genes are removed from the MoMuLV vectors, and the functions normally provided by these deleted sequences are made available through the use of "packaging" lines. Despite the many advantages, there are a series of limitations when using retroviral vectors. First, these viruses can transduce only dividing cells. Second, retroviruses can stably integrate into the host genome, but this is a random event and opens the possibility for "insertional mutagenesis" that can lead to the development of a malignancy in the treated subject. Another disadvantage seems to be the very low growth titers of retroviruses in cell culture. Furthermore, recent reports on retrovirus-transduced bone marrow in monkeys have raised some debate regarding the safety aspect of this delivery system.

Adenoviral vectors have been developed primarily for gene transfer into non-dividing cells. These viruses (types 4 and 7) have been extensively used for the vaccination of US military recruits and have demonstrated a high degree of safety for human use. They are able to infect only quiescent cells and do not integrate in the host genome. This means that transferred genes are eventually lost from dividing cells. Thus, if a long-term effect is required, patients will have to undergo repeated treatments and it is yet unclear whether repeated administration of the adenoviral vector could cause undesirable side-effects in the target recipients.

However, if therapy is targeted to slowly dividing tissues, such as lung or liver, two or three applications a year might be sufficient.

Vectors derived from the non-pathogenic human adeno-associated virus (AAV) are also used for gene transfer. These viruses are known to integrate in a small region of the human genome, on chromosome 19. Their production requires the use of "helper viruses", such as adenoviruses or herpes viruses. Thus, there is always a possibility that the AAV vector stock could carry contaminating traces of the pathogenic helper viruses and this could raise some safety concerns.

Herpes simplex virus type 1 (HSV-1) has been developed recently for gene delivery into non-dividing neurons. Similarly to the adenovirus vectors, HSV-1 vectors will not integrate into the genome. The possible pathogenicity of this vector is as yet unknown.

Several non-viral methods may provide attractive alternatives to viral vectors for gene therapy. The most promising procedures in this category are receptor-mediated gene delivery and liposome mediated gene transfer. Transferrin-receptor-mediated gene transfer of DNA conjugated with transferrin-polylysine has proven effective for *in vitro* transduction of non-dividing cells.

Oligonucleotides present interesting challenges for delivery systems since they are particularly susceptible to degradation by nucleases in the biological milieu and usually cannot cross the target cell membrane. The potential of liposomes to encapsulate antisense oligonucleotides or DNA, protecting them from degradation, represents a great advantage over other drug carriers. Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shells of phospholipids. The potential use of these substances as drug carriers was recognised more than 25 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications. One of the limitations of these compounds is that they have a short lifetime. However, recently, a significant advance has come with the incorporation of specialised lipids, such as monosialoganglioside GM1 or polyethylene glycol modified phosphatidyl ethanolamine, that increase the circulation lifetimes of liposomes. It has been demonstrated that increased circulation lifetimes enhance the opportunity for liposomes, administered systematically, to leave the vascular compartment and enter, for example, tumours.

Results from a phase I clinical study on cationic liposome-mediated cystic fibrosis transmembrane regulator (CFTR) gene transfer to the nasal epithelium of patients with cystic fibrosis have recently been reported (Caplen *et al.*, 1995). No adverse clinical effects were observed from gene transfer to the nasal epithelia. The next generation of liposomal pharmaceuticals will consist of drug-loaded liposomes with surface-associated targeting information that will direct them toward specific cells.

Another procedure, which has led to stable gene transfer in muscle but not in other tissues, has been, as already described, the direct injection of naked DNA. Related to this procedure is the development of the so-called particle bombardment technology using the "gene-gun". Several tissues in mice, including skin, liver and muscle, can be successfully transduced with this new technology.

It is evident that much of the technology on delivery systems available today takes advantage of the knowledge derived from the development of viral vaccine vectors. Thus, many of the safety concerns and guidelines raised for the new live viral vaccines could be raised for the gene-therapy vectors, as the recent OECD meeting on the subject ("Gene Delivery Systems", Ottawa 1995) indicated.

VIII. THE NEED FOR NEW MODELS FOR HUMAN DISEASES

The use of animal models for human diseases has been the focus of heated debate for many years. Why is it so important to take advantage of animal models in the study of human diseases? One of the principal reasons is that for any new medical intervention, issues of safety and efficacy are paramount. Consequently, animal models are used to evaluate and optimise experimental therapies for which *in vitro* studies provide only limited information. However, many questions are still raised on the validity of this argument. In particular, sceptics point to differences in development and behaviour between humans and other animals. In view of this, an important distinction should be made between animal phenocopies of a human disease and a true genetic counterpart. An animal phenocopy could show symptoms (or a phenotype) similar to a human disease without actually having a similar genetic or biochemical disorder. On the other hand, a true genetic counterpart will have been selected to carry a human genetic disorder: these animal models are genuine genocopies of human diseases and are the only ones suitable for testing treatments for many metabolic and genetic disorders.

The development of transgenesis and gene targeting in embryonal stem (ES) cells in mice has opened the path to the production of an endless number of genocopies of human diseases. Any human condition for which a gene has been characterised and cloned can, theoretically, be generated in the mouse and indeed, in the past few years, a significant number of human diseases have been modelled in the mouse. Thus, the mouse now plays a vital role in the development and testing of new therapies. Comparative genetic mapping has recently revealed a striking degree of gene and linkage conservation between humans and mice. This means that many disorders in humans and mice display similar phenotypical features and modes of inheritance, and often map to gene clusters that have been conserved throughout evolution. This fact, together with the knowledge available

on the genetics of the laboratory mouse, emphasizes the relevance of this animal for the study of human diseases.

In general, mutations that cause a gain of function produce disease even when they occur in only one of the gene's two alleles. In a recessive genetic disorder, by contrast, there must be mutations in both alleles for the disease to be produced, and the mutations cause a loss of function. The methods needed to produce animal models of recessive genetic diseases differ from those used in studying autosomal dominant diseases. To create an animal model of the former class of diseases, both alleles of the normal gene must be inactivated. The technique of gene "knockout" was developed for this purpose. More recently, gene-targeting (knockout) was developed to replace the specific gene of interest with one that is either inactive, altered or nonsense. Further recent improvements in knockout technology include the ability to inactivate a gene at a specific time after conception and in a specific tissue using a bacteriophage site-directed recombination system (Cre-lox) consisting of site-specific recombinase (Cre) and DNA recombination sites (lox). Currently, many human disorders have been modelled and are being studied in knockout mice (Table 4). Pharmacological manipulation of knockout mice is very useful in screening therapeutic agents with potential for study in clinical trials. More importantly, somatic gene therapy and new delivery systems can be tested in a disease model using knockout mice. An example of this is adeno-virus mediated gene transfer of the receptor for low-density lipoprotein (LDL) into LDL-receptor-deficient knockout mice, which results in partial amelioration of the phenotype.

It is important to note that the extensive use of this animal to generate disease models has given rise to some concern, and various OECD countries have now developed guidelines for the production of transgenic animals. Although it is likely that most of these animals will contribute substantially to the effort to eradicate diseases, the development of transgenic mice can host human pathogens such as viruses or prions may create special public health concerns. The risks posed by transgenic animals harbouring receptors for human pathogenic viruses were the subject of a recent debate at the World Health Organisation. On this occasion, it was recognised that, for example, transgenic mice expressing the poliovirus receptor, and thus susceptible to poliovirus infection, could, in theory, become a new reservoir and source of the virus in the environment. Thus, even if the risk of the "escape" of a transgenic mouse is very low, it is essential to follow safety recommendations concerning the maintenance, containment and transport of transgenic animals susceptible to human viruses or other human pathogens.

The techniques of gene replacement and knockout represent major advances in biology and pharmacology, and many hundreds of mutant strains have already been created for both academic and industrial research. Thus, there is an urgent and growing need for well-managed "archives" in which mutant mice

Table 4. Examples of human disorders studied in transgenic mice

Cardiology
Atherosclerosis
Salt-sensitive hypertension
Endocrinology
Familial hypocalciuric hypercalcemia
Glycogen storage disease type 1
Obesity
Growth retardation
Gastroenterology
Hirschsprung's disease
Ulcerative colitis
Hematology
α-Thalassemia
Hemophilia A
Chronic granulomatous disease
Immunology
Autoimmune lymphoproliferative syndrome
Bruton's agammaglobulinemia
Hyper-IgM syndrome
Severe combined immune deficiency
(Autosomal recessive; X-linked)
Metabolism
Gaucher's disease
Homocysteinemia
Lesch-Nyhan syndrome
Neimann-Pick disease
Tay-Sachs disease
Neurology
Short-term memory deficit
Oncology
Li-Fraumeni syndrome
Neurofibromatosis
Retinoblastoma
Pulmonology
Cystic fibrosis

Source: Adapted from J.A.Majzoub and L.J. Muglia (1996), *The New England Journal of Medicine*, Vol. 334, No. 14, pp. 904-907.

and/or cryopreserved sperm or embryos can be reliably stored and documented. The choice of the location of such repositories, the type of arrangements for the diffusion and sharing of the strains, and the associated information, rights and obligations, raise important policy issues that are currently being considered by the European Commission and by the OECD.

IX. CONCLUSIONS

At a time when governments are cutting down on research and health spending, new alliances among venture capitalists, drug companies, academia and medical enterprises are changing the traditional relationships between research, industrial development and clinical practice. An "integrated" industry model is replacing the pharmaceutical model of the 1970s and 1980s. This change was already set in motion in the early 1970s, when the sharp division between medicine and biology started to blur and with the development of biotechnology companies with a new vision, such as Syntex and Genentech.

However, it is more recently that biotechnology firms and pharmaceutical companies have recognised their mutual benefit in developing strategic alliances. Biotechnology firms need the support of pharmaceutical companies that have the resources and the potential to develop the studies necessary for registration filing and commercialisation, and that have a marketing and distribution network. Pharmaceutical companies, on the other hand, benefit from acquiring on an *ad hoc* basis new technology and competence that they do not have "in house" and that would require major restructuring. Pharmaceutical companies ultimately also benefit from the global commercialisation of competitive new products. Since the beginning of this decade, drug firms have formed more than 200 strategic alliances with small biotechnology firms. Thus, the boundaries between biotechnology research and major pharmaceutical company R&D are fading as biopharmaceutical companies integrate their activities into the larger corporations.

Despite these strategic solutions to risk sharing and the new stunning technical achievements of the biopharmaceutical industry, the road to commercialisation is not easy for most biopharmaceutical products. PMA estimates that it takes on average US$231 million and 12 years to bring a pharmaceutical product from early stage research to the market: scientific research, animal and clinical testing are time- and resource-consuming.

The aftermath of the long and expensive road to commercialisation is ultimately reflected in the elevated cost of new "breakthrough biopharmaceuticals". From a public expenditure perspective, pharmaceutical products represent a small fraction of overall health-care costs (10-25 per cent), and the new drugs are meant to cure and/or prevent some of the most untreatable diseases. Thus, in the long term, they are expected to produce an overall reduction of health-care expenditures. However, in the current situation of general concern for the escalation of health-care expenditures, innovative technologies and the new biopharmaceuticals are very likely to experience increasing presssure to justify their existence and development on economic grounds.

These considerations raise questions about the current pharmaco-economic evaluation criteria. Should the new drugs be priced and reimbursed in relation to

the therapeutic improvement that they may offer when compared to a given traditional drug? Should pharmaceutical companies be prepared to demonstrate the "socio-economic" value of a new drug? Evidently, the problem is that "new medicine is not enough", improved medicine is what we are really seeking. However, this concept needs to be better defined, and any amendment will inevitably also involve ethical and philosophical issues. The problem is that pharmaco-evaluation criteria can include analyses of direct cost/benefit as well as analyses of wider economic gains and less tangible benefits, such as the often unpredictable, and yet fundamental, gain in "quality of life". Thus, there is a need to formulate clear health-care policies for the future which will require new thinking on the part of governments with regard to how to measure research and health-care priorities; how to evaluate technology; and how to set pricing and reimbursement policies.

As a final point, it is important not to dismiss an even more fundamental question: "What are the conditions that would make the new health technologies an effective response to the needs of the public, and how can they be practised?" People must want to protect themselves from infection or diseases, must be aware of what their options are and must be able to practice them. Thus, in a world of changing notions about the therapeutic approach to diseases, education and easily accessible health information are of paramount importance.

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INTELLECTUAL PROPERTY IN HUMAN GENOME RESEARCH RESULTS

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This article was written by Professor Joseph Straus. Professor Straus is Professor of Law and Head of Department at the Max Planck Institute for Foreign and International Patent, Copyright and Competition Law in Munich; he teaches German and European patent law at the Faculty of Law, University of Munich and, as Visiting Professor of Law, international protection of intellectual property rights at Cornell Law School, Ithaca, NY. He chairs the Intellectual Property Rights Committee of the Human Genome Organisation (HUGO) and the Biotechnology Committee of the International Association for the Protection of Industrial Property (AIPPI). Professor Straus has acted as consultant to a number of international organisations, including the OECD and the EC Commission. He has extensively published in the area of intellectual property law, especially on the protection of biotechnological inventions. The views expressed in this contribution, however, are his own and sole responsibility.

I. INTRODUCTION

The priority claim for having initiated an international debate on patent protection in biotechnology is clearly with the OECD. The OECD, through its 1985 report *Biotechnology and Patent Protection – An International Review,*[1] provided a first inventory of respective statutory provisions, case law and administrative practices, based on replies from OECD Member countries to a questionnaire. In view of the established differences in national laws, the OECD group of experts strongly emphasized the need for further steps to be taken towards international harmonization of patent law in this rapidly developing area of science and technology, and submitted a number of general as well as specific recommendations to the governments of OECD Member countries for consideration.

However, neither the general focus of the OECD questionnaire, nor the responses of the Member countries, nor the recommendations of the expert group foretold the legally and socially intriguing issue of intellectual property in genome research results, which sparked off only some six years later and which shook up the scientific community involved in genome research as well as the general public. In June 1991 the US National Institutes of Health (NIH) filed with the United States Patent and Trademark Office (US-PTO) an application which claimed protection for 3 421 cDNA sequences of unknown function containing a total of 724 837 nucleotides, detected in the course of research performed in the framework of the Human Genome Project, a publicly funded ambitious endeavour, which started in 1987 and which is aimed at mapping and sequencing the entire human genome. This may not surprise, since the idea of mapping and sequencing whole genomes, in particular all of the estimated 3 billion base pairs of the human genome, was clearly beyond imagination at the time when the OECD initiative was launched. Only with the advent of improved automatic DNA sequencing and DNA amplification by polymerase chain reaction (PCR), as well as other advanced techniques, did the most courageous optimists in the second half of the 1980s begin to consider such an attempt as a realistic goal.

II. GENES AS PATENTABLE SUBJECT MATTER

The OECD questionnaire did not directly address the issue of patent protection of genes. Since, however, genes (*i.e.* the fundamental physical and functional units of heredity in its broadest sense), consisting of an ordered sequence of nucleotides located in a particular position on a particular chromosome and which encode a specific functional product, such as a protein or an RNA molecule, are to be regarded as biochemical substances, and thus inanimate products of nature, one question of the questionnaire rather indirectly addressed the issue: Member countries were asked whether their patent laws protect "valuable non-living material related to or created from natural sources, *e.g.* new chemical substances such as vectors which are essential intermediates in biotechnology?". The replies reflected, on the one hand, the old patent law dilemma of all products of nature involving the distinction between unpatentable discoveries and patentable inventions and, on the other, the fact that at that time even in some OECD Member countries no product protection for chemical substances existed. Moreover, the replies made it quite clear that the patent offices of most Member countries had at that time virtually no experience in patenting genes, and only limited experience in patenting gene transfer vectors. Thus, the responses of Belgium, France, Germany, Ireland, the Netherlands and the United Kingdom confirmed the patentability of transfer vectors assuming these to be new chemical substances, *e.g.* novel plasmids, with Belgium, France and Germany also noting that there is nothing to prevent the patenting of inanimate natural products provided that there is human intervention, necessary to arrive at the product. However, only Germany reported court decisions affirming the patentability of naturally occurring substances. The group of experts concluded and suggested:

"Such products as genetic engineering vectors seem to be patentable in OECD countries which accept product protection in general. It is suggested that the patentability of genetically engineered inanimate products should be accepted in other countries as well."[2]

The Examination Guidelines of the European Patent Office (EPO) from the outset treated naturally occurring chemical substances along the same lines: neither the fact that such discoveries are not regarded as inventions under the European Patent Convention (EPC), nor the novelty requirement, posed insurmountable obstacles for their patentability. According to the Guidelines (Part C-IV 2.3), a substance found in nature which must first be isolated from its surroundings and can be properly characterised either by its structure, by the process by which it is obtained, or by other parameters, and is "new" in the absolute sense of having no previously recognised existence, can be patentable *per se,* provided the inventor discloses the manner of how to obtain it in a repeatable way, and

provided, it goes without saying, that the patentability requirements of inventive acitivity (non-obviousness) and industrial applicability (utility) are met.

Subsequently and practically unnoticed by the general public, and with no objection from the scientific community, patent offices of industrialised countries and the European Patent Office issued patents not only on gene-transfer vectors, but also on DNA sequences – genomic as well as cDNA sequences – coding for a number of pharmaceutically important proteins, such as erythropoietin, various interferons, human growth hormones, granulocyte colony stimulating factors, tissue plasminogen activator, various interleukins, tumour necrosis factor, etc. According to the data collected by S.M. Thomas *et al.*,[3] between 1981 and 1995 a total of 1 175 patents for human DNA sequences were granted world-wide, most falling in the category of anti-tumour/antiviral.[4] These patent granting practices have not since been put into question by national courts. A whole new biotechnology industry emerged predominantly in the United States, the two main assets of which were patent protected inventions on the one hand, and the skills of their inventors (mainly academic scientists – molecular biologists, geneticists, physicians and others involved in research in life-sciences), on the other hand. The latter merged their skills with those of professional managers, who were able to attract venture capital and take care of organisational as well as commercial matters. In 1982 the first drug, human insulin, produced by rDNA technology came onto the world market. At the end of the 1980s/beginning of the 1990s, the first two block-buster drugs, Epogen (erythropoietin) and Neupogen (GCSF), followed, generating a world-wide turnover of more than US$3 billion by the year 1995. Needless to add, patents on DNA sequences coding for the proteins at stake were instrumental in attracting the necessary investments and thus in the success of the inventors and to the benefit of patients. It should, however, also be added that the respective patents are still under attack from a number of competitors, with litigations pending in a number of important industrialised countries.

After sometimes controversial debates, the leading US research universities decided in favour of an active patent policy, and put in place the infrastructure necessary for acquiring, upholding and defending patents as well as for the successful commercialisation of research results: they now hold 18.1 per cent of all patents issued in the area of genetic engineering[5] and, together with the US Department of Health and Human Services, more than half of all US-PTO human DNA sequence patents.[6] In 1995, for instance, the University of California system ranked number 3 among the applicants in the field of biotechnology in the European Patent Office. The same attitude has been advocated by the US Department of Energy (DOE), which has co-sponsored the Human Genome Project (HGP) from its initiation. From the beginning DOE has favoured a wide application of the patent mechanism to protect the rights to inventions, and to open up the transfer of knowledge and inventions for the public good.[7]

While it certainly cannot be claimed that these developments were well received by all members of the scientific community, it remains true that they neither adversely affected genome research nor did they provoke substantial negative reactions on the part of the general public. This merits particular attention in view of the fact that even the basic technology of inserting foreign genetic material into bacterial plasmids, developed by Cohen and Boyer, has been patented in the United States for the University of Stanford and the UC San Francisco. The subsequent policy of the patent owners to licence this basic technology on a non-exclusive basis and on very liberal terms, however, prevented negative reactions from those who heavily depend on the technology, *i.e.* industry and academia, as well as the general public.[8]

III. PATENTING OF GENOME RESEARCH RESULTS IN NEW PERSPECTIVE

The NIH application of June 1991 triggered a whole range of reactions, reflecting both a number of legal issues, which had to be resolved by the US-PTO for the first time, and the general concern of the scientific community, as well as industry and even politics,[9] on the possible negative consequences for research, development and international co-operation if patents were to be granted on partial or full-length cDNA sequences with as yet unknown functions, the so-called Expressed Sequence Tags (ESTs). Since the resignation of Nobel laureate James Watson from his post as Director of the US National Human Genome Center in the wake of the NIH ESTs application has often been linked to the issue of patenting human DNA sequences in general, it seems essential to clarify the fact that most prominent researchers in the field, including James Watson, Paul Berg, Walter Gilbert (both Nobel laureates), Sir Walter Bodmer, Sydney Brenner, Leroy Hood, Eric Lander and Francis Collins, the successor of James Watson as Director of the US National Human Genome Center, to name but few, have always demonstrated a positive attitude towards patenting of full-length cDNAs with known functions, but have strongly opposed patents in ESTs with as yet unknown useful functions. These observations are very well reflected in a report published by the Science and Technology Committee of the UK House of Commons on "Human Genetics: The Science and Its Consequences" in which it is expressly noted:

> "We note the general agreement among our witnesses, including pharmaceutical companies and the Bioindustry Association, that fragments of genes, or genes of no known function, should not be patentable. We conclude – only a combination of a gene and a known utility which is novel and not obvious

should be patentable in the context of that utility; and a combination of the same gene and a further novel utility should also be patentable."[10]

In order to do justice to NIH, prior to analysing the specific legal and social concerns which its application has provoked, some explanation of the background of this application and the motives of its filing is necessary. As has been correctly pointed out, the basic problem which the NIH application had to face from the outset was a direct result of the programmatic decision of the NIH researcher Craig Venter and his group at the National Institute of Neurological Disorders and Stroke to characterise the human genome through a large-scale (*i.e.* sequence-based) approach as opposed to the traditional functional approach.[11] Whereas in the latter method, which involves a number of cumbersome steps, at least one function of the given gene is always known, this, by virtue of the approach, is not true for the first method. This lack of knowledge on functions of full-length or partial cDNAs, however, does not automatically deprive the sequences of any commercial value. Bound by federal technology transfer laws and policies, NIH felt obliged to file the application in order to facilitate technology transfer, *i.e.* to enable industry as a potential partner to seek licences and subsequently develop marketable products from the acquired information. At the same time as the application was filed, the entire sequence information was released to the public. Without patents on these cDNA sequences, in the opinion of NIH the published sequence information might have seriously threatened the possibility of patenting future products and thus reduced the readiness of industry to invest in development in this area. NIH, however, realised the problems involved and suggested that amending the obviousness requirement of the patent law would be salutary. Moreover, improvements as to the effectiveness of use patents at an international level were seen as a possible means of ensuring adequate protection for sequence-related inventions in world markets.[12]

US lawyers who critically analysed the possible impacts of patents issued in ESTs observed that if claims were to solely concern ESTs *per se* – strictly *per se,* in other words claims directed to a DNA consisting of the EST – such patents might inhibit others from actually copying the disclosed EST sequence and using it or portions of it as a probe, but no more. No problems, and thus no dependencies, would emerge from such patents for almost any independent research which did not rely on the disclosure of the NIH itself. If, however, claims were to be granted to the entire gene of which the EST is a part, including portions of that gene, or even degenerate forms of the coding sequence in that gene, then this would prevent others not only from using materials discovered independently for purposes of probes, antisense protocols and the like, but also from producing the recombinant protein encoded by the gene of which the EST is a part.[13]

The genome community world-wide, as well as industry, rejected the idea of having patents on ESTs without known functions as this would represent an

untenable level of monopolisation of research results. The genome community also expressed most serious fears that such patents would damage international co-operation, essential in this important area of research. In particular, human genome researchers performing large-scale mapping and sequencing of the human genome, who collaborate internationally under the umbrella of the Human Genome Organisation (HUGO) with publicly or charitably funded research efforts exceeding US$250 million per year, clearly expressed their views: in a position statement on cDNAs: Patents of 6 January 1992, HUGO declared that it does not, in general, oppose patenting of useful benefits derived from genetic information. It added, however, expressly that it does:

> "oppose the patenting of short sequences from randomly isolated portions of genes encoding proteins of unknown function. While the EST is a useful tool in the conduct of further research and, as such, is being used in many projects all over the world, it is HUGO's belief that approval of EST patents might imply a significant change in the standard of scientific discovery required for a patent, since it is hard to accept the proposed scientific definitions of novelty and utility for the EST work..."

In a further statement in April 1995, HUGO expressed its concern that the patenting of partial and uncharacterised cDNA sequences would reward those who make routine discoveries but penalise those who determine biological function or application. In HUGO's view such an outcome would impede the development of diagnostics and therapeutics, which would clearly run against the interests of the public. The statement concluded that it would be ironic and unfortunate if the patent system was to reward the routine while discouraging the innovative. Yet that could be the result of offering broad patent rights to those who undertake massive, but routine, sequencing efforts – whether for ESTs or for full genes – while granting more limited rights or no rights to those who make the far more difficult and significant discovery of underlying biological functions. In this HUGO statement, which again acknowledged the role of patents as a necessary incentive for the ongoing development of products in this area, another important aspect was emphasized: it would be equally unfortunate if a partial sequence publication or submission to a database precluded the patenting of innovative disease gene discoveries leading to improved medical diagnostics and therapeutics. This could lead to inhibition of contributions to databases and lack of investment protection for the innovative. Therefore, HUGO expressed the hope that "the system will find some way to adjust to the changing realities in this field to promote and protect this important and ongoing process of discovery in the public interest."[14]

From the legal point of view, doubts have been expressed from the outset as to how ESTs of unknown function could comply with the patent requirement of utility. The NIH specification tried to meet these requirements by indicating that

ESTs may be used as probes to isolate coding sequences and complete genes, which may then be mapped to chromosomal locations. Also, the suggestion was made that ESTs can be used as chromosome markers, for forensic identification, or for tissue typing. Eventually the US-PTO in December 1992 rejected the NIH application due to lack of utility (35 U.S.C. § 101) of most claims and also in some instances due to lack of novelty [35 U.S.C. § 102 (b)]. The examiner emphasized that, in view of the disclosure in the instant application, it would be necessary to do further work to establish a utility for any of the nucleic types embraced by the claims. As the fierce debate continued, the NIH Director, Nobel Prize winner Harold Varmus, announced in February 1994 the NIH decision to withdraw all its patent applications for ESTs relating to all-in-all 6 869 sequences. Varmus declared that patents on such partial sequences are "not in the best interest of the public or science."[15] MRC followed suit. Since private companies, such as Human Genome Sciences (HGS) of Gaithersburg, MD, and Incyte of Palo Alto, CA, which have filed for 9 900 and 40 000 sequences, respectively, did not withdraw their applications pending in the US-PTO and the EPO, the issue remains unresolved for the time being.

The European situation differs in at least one important point from US law: under the European Patent Convention's (EPC) requirement of industrial applicability (Art. 57), corresponding to the utility requirement under the US law, an invention is regarded as industrially applicable if it can be made or used in any kind of industry, including agriculture. Since there is no doubt that ESTs can be produced and even used in industry, Art. 57 EPC alone cannot prevent their patenting. Nonetheless, apart from the patentability requirements of novelty and inventive step (non-obviousness) which, due to the ever increasing amount of sequence information in the public domain, on the one hand, and the continuous progress of sequencing techniques involved, on the other, have become high hurdles to overcome, another objection to the patentability of ESTs has been convincingly raised: if no other functions are known regarding ESTs than those indicated in the NIH application, then the key question for assessing patentabilty is whether the ESTs solve a technical problem. It can be argued that an EST invention does not solve a technical problem if it does not disclose more than the provision of human genes probed by the ESTs. However, these genes have not yet been provided and their functions remained unknown.[16]

IV. GENERAL ETHICAL CONSIDERATIONS

Whereas the reseachers involved in genome research, interested industry, patent offices and courts, as well as large parts of the general public, had no problem in accepting patents claiming full-length DNA sequences with known

functions, regardless of their origin, as an important means of safeguarding the necessary investments in drug development and as a just reward for the achievements of scientists, some influential groups in the United States, as well as in Europe, have raised their voices against patents on all life-forms, including patents on genes.

The latest widely publicised attack against patents in living matter in the United States was orchestrated by Jeremy Riffkin's "Foundation for Economic Trends", together with the "General Board of Church and Society" of the United Methodist Church (UMC) and a number of other organisations. The so-called "Blue Mountain Declaration" of 2 June 1995, which was signed by a large number of religious leaders, reads in this regard *inter alia* as follows:

> "The humans, animals, micro-organisms and plants comprising life on earth are part of the natural world into which we were all born. The conversion of these life-forms, their molecules or parts into corporate property through patent monopolies is counter to the interests of the peoples of the world.
>
> No individual institution, or corporation should be able to claim ownership over species or varieties of living organisms. Nor should they be able to hold patents on organs, cells, genes or proteins, whether naturally occurring, genetically altered, or otherwise modified."[17]

However, this view is by no means shared by all theological institutions. On 25 September 1995, Ted Peters, Professor of Systematic Theology and Head of the Centers for Theology and Natural Sciences (CTNS), Berkeley, research project on "Theological and Ethical Questions Raised by the Human Genome Initiative" reacted vigorously and called on religious leaders not to follow the "Pied Piper of the coalition against life patents", because: arguments that oppose patenting on the grounds that DNA or natural life-forms are in themselves sacred and inviolable were theologically unwarranted; other far more pressing theological issues related to gene research should command the intentions of religious leaders, and to syphon off religious energy into the patenting controversy was to be viewed as bad stewardship; and because the patenting controversy consisted of an ideological and economic battle in which the churches have no vested interested in who wins." In his declaration, Peters further stated *inter alia:*

> "There is nothing in the Bible that would warrant us to say theologically that our genetic code as found in the DNA represents God's final will for human well-being. DNA is part of God's creation, to be sure: but DNA too is fallen. On occasion our genetic code builds suffering right into human experience: perhaps as many as 4 000 diseases are due to genetic predispositions which are inborn. Some persons are born with better genomes than others, leading those who suffer from horrible diseases such as cystic fibrosis to raise their

fists against nature crying 'Injustice!'. Should we blame God for building injustice right into nature?

That scientific research into human genetics may eventually provide therapy for cystic fibrosis and similar conditions and dramatically reduce human suffering constitutes God's ongoing redemptive work through human agency. To say that DNA as we currently find it is inviolable and should remain in the way it is without human intervention is to thwart this significant redemptive work.

Patenting belongs to the research process. The prospect of patent protection draws venture capital – perhaps US$1.5 billion this year – to fund research into inventing medicines and therapies that are bringing leap-frog advances in the quality of human health. When opposing the patenting of intellectual property regarding genetic knowledge, ethically minded people need to think seriously about the consequences of retarding or stopping the advance of medical research. This in itself is not a sufficient argument for keeping the current patenting policy, to be sure, yet the ethical gravity of this factor must be considered in any anti-patenting arguments.

The current practice of the Patent and Trademark Office is to approve patents that exhibit 'novelty' – that is, the quality of human intervention and human creativity. Patent protection is not granted for nature as it already exists – that is, not for DNA sequences as nature has provided them. Nor are patents granted for living persons or their body parts as we find them in a state of nature. Because of the sophisticated status of genetic research the line between what exists already in nature and what human ingenuity invents is a blurry one, to be sure. Yet, it can be said with reasonable accuracy that the PTO is not in the business of granting profit to selected companies just because they hold patents on God's creation prior to the advance of human novelty."

After identifying genetic discrimination in employment and insurance, the increase in selective abortion of foetuses with genetic defects, the philosophical issue of genetic determinism against human freedom and germ-line intervention as the four major ethical issues of genome research, Peters concluded:

"These are social and political issues that the scientists in their laboratories cannot solve by themselves. It will take the best minds among our philosophers and political leaders – and, yes, our religious thinkers – to come to the kind of ethical understanding that could lead to healthy public policy. For religious leaders to get excited about the patenting controversy and then assume that they have made their contribution to social justice would constitute a form of blindness in the middle of seeing."[18]

Other theologists,[19] leading scientists,[20] as well as philosophers[21] argue along basically the same lines.

In contrast to US patent law, which does not dispose of any provision which would link the patentability issue of an invention to any specific consideration of ethics or morals, Art. 53(a) EPC and the corresponding provisions of national patent laws of European countries exclude from patenting inventions, the publication or exploitation of which would contradict *ordre public* or morality. Despite this provision, the European Patent Office (EPO) never considered it necessary in the past to apply ethical considerations with regard to claims for human genes. This is because patents can only be granted in relation to technical inventions and their effect is restricted to excluding others from using the genes in an industrial process and, thus, does not affect in any way the personality of human beings. The first case in which the EPO had to tackle this problem was in the opposition proceedings against a European patent relating to human H2-relaxin, in which the opposing Green Party in the European Parliament contended that the subject matter of the disputed patent, insofar as it related to a DNA fragment encoding human H2-relaxin and its precursors, offends morality. The isolation of the DNA relaxin gene from tissue taken from a pregnant woman was considered immoral in that it constituted an offence against human dignity to make use of particular female conditions (pregnancy) for a technical process oriented toward profit. Thus, it was argued, the patenting of human genes such as that at issue amounts to a form of modern slavery since it involves the dismemberment of women and their piecemeal sale to commercial enterprises throughout the world; the patenting of human genes means that human life is being patented. The EPO Opposition Division rejected the opposition and made the point that patents covering DNA encoding, for instance for H2-relaxin or any other human gene, do not confer on their proprietors any rights whatever to individual human beings, no more than do patents directed to other human products such as proteins. No woman was affected in any way by the patent. Since the protein encoded by the cloned gene is produced in a technical manner from unicellular hosts containing the corresponding DNA, there is therefore no need to use human beings as a source for a protein. The EPO added that DNA is not "life" but a chemical substance which carries genetic information, and can be used as an intermediary in the production of proteins which may be useful medically. The Opposition Division saw no moral distinction in principle between the patenting of genes on the one hand, and of other human substances on the other, especially in view of the fact that only through gene cloning have many important human proteins become available in sufficient amounts to be medically applied.[22]

The opposition of parts of the European Parliament to the patentability of human genes is also well-reflected in the fate which the Proposal of the EC Commission for a Council Directive on the Legal Protection of Biotechnological Inventions has experienced since the initiative started in 1988.[23] Primarily because under the proposal eventually discussed in the European Parliament

patents on isolated human genes and human gene therapy, even germ-line therapy, were in principle allowed, the European Parliament dropped the proposal on 1 March 1995. While the new proposal submitted by the Commission in December 1995[24] on the one hand explicitly excludes from patenting "methods of human treatment involving germ-line gene therapy" as contradicting *ordre public* or morality [Art. 9(2)(a)], it, on the other hand, explicitly declares that, despite the exclusion from patentability of the human body and its elements in their natural state, "the subject-matter of an invention capable of industrial application which relates to an element isolated from the human body or otherwise produced by means of a technical process shall be patentable, even if the structure of that element is identical to that of a natural element" [Art. 3(2)]. Therefore, genomic as well as cDNA full-length sequences for which a useful function can be indicated, should remain patentable under this proposal.[25]

V. POLICY CONSIDERATIONS

It goes without saying that a distinction has to be made between legal issues under the law in force, legal policy considerations, which means considerations policy makers have to make when reviewing the effects and impacts of the laws in force and comparing them with the desirable results, and policy considerations of those directly involved.

As far as policy makers' considerations are concerned, nothing specific can be reported for the United States or Japan. The project of the former OTA on "Patenting Human Genes and the Human Genome Project" ended in a background paper on "Federal Technology Transfer and the Human Genome Project"[26] which, although it offers a number of interesting data, does not reflect its original goal and does not even touch upon the patentability issue of ESTs and the possible impacts of such patentability. The plans of the European law maker, which are at present *de novo* discussed in the European Parliament, as far as they are of interest, have been already indicated. What remains to be addressed here is a short review of the policies of those directly involved in human genome research, which means academics loosely collaborating and planning under the umbrella of HUGO on one side, and industry, on the other.

As far as the work of publicly or charitably funded researchers collaborating under the HUGO auspices is concerned, it should be emphasized that this work has been guided from the very outset by open access and generous data-sharing policies. Even competing groups mapping and sequencing important chromosomes, such as chromosome 21, the involvement of which in Down's Syndrome was already known and that in Alzheimer's disease assumed, worked closely together and exchanged their complete data.[27] Many leading researchers heading

the centres for large-scale sequencing, such as the Sanger Centre in Cambridge, United Kingdom, (John Sulston), or the Human Genome Project Center at Washington University in St. Louis (Robert Waterston), have the reputation of being paragons of collaboration, who have always advocated the immediate release of all sequenced data.[28] Although this attitude has in principle always been advocated by HUGO, in the past the funding agencies did not impose any formal constraints on the grantees. However, this attitude has recently changed. At a meeting convened by the Wellcome Trust in February 1996 in Bermuda, in which scientists from genome centres in the United Kingdom, the United States, France, Germany and Japan participated, an agreement was sought for some general principles on mechanisms of co-ordination and potential scenarios for data release. It was finally agreed that all human genomic sequence information, generated by centres funded for large-scale human sequencing, should be freely available and in the public domain in order to encourage research and development and to maximise the benefits to society. It was also agreed that sequence assemblies should be released as soon as possible and that finished, annotated sequences should be submitted immediately to the public databases. The idea underlying this agreement was that human genomic sequences generated by large-scale sequencing centres, funded for the public good, should be immediately released in order to prevent such centres from establishing a privileged position in the exploitation and control of human sequence information. At the same time, an agreement was also reached on the promotion of the co-ordination of sequencing activites: large-scale sequencing centres should inform HUGO of their intention to sequence particular regions of the genome. This information will be presented on HUGO's World Wide Web page which will direct users to the web pages of individual centres for more detailed information regarding the current status of sequencing in specific regions. These mechanisms are aimed at enabling centers to declare their intentions in a general framework, while also allowing more detailed interrogation at the local level.[29] The participants of the Bermuda meeting were of the opinion that access to the initial genomic sequence as it is generated will provide maximum opportunity for research and for development of new products. It seems that these principles have been fully reflected in the NIH funding policies within their legal limits. The National Center for Human Genome Research is testing its own powers to persuade investigators to share information. New grantees will be required to release all their sequence data on the World Wide Web "within a few days or weeks" of discovery. New grantees are also encouraged not to take out patents on raw sequence data.[30]

Whether the sequence data release policy announced in Bermuda will be applied by all those involved in human genome mapping and sequencing activities remains to be seen. Smaller groups, who wish to present complete data and also, if appropriate, seek patent protection, seem to have problems with this

policy. Also, it is indispensable to study cautiously all the possible impacts of such a far-reaching move. While it sounds plausible, and is most probably as a rule correct, that the accessibility of the sequenced raw data will not generally prevent the patenting of the results of subsequent research, especially of diagnostics and therapeutics based more or less directly on such data, and does not pose serious obstacles for the necessary investments, other important issues are still awaiting competent answers. Bearing in mind the differences which exist, for instance, between the US patent law and the EPC as regards the novelty requirement, it should be noted that patents on such published data, provided that the data meet the patentibility requirements, could still be obtained in the United States, thanks to the existing "grace period" of one year allowing successful filing up to one year after publication, but not in Europe.[31] Moreover, and this is less a legal than a science policy consideration, the Bermuda "agreement" would inevitably divide researchers involved in human genome research into two large groups: the mappers and sequencers, asked to immediately publish the raw sequence data, on the one hand; and "the hunters" for disease and other relevant genes, who will be hunting on the pastures provided by the first group and who will, for sure, try to patent their "bag". Whether the genome research community will be willing to accept a situation in which their huge endeavours to map and sequence the human genome will put other researchers, the hunters, in a position to talk "about doing 'virtual cloning' and 'armchair genetics' because they can use libraries of DNA to deduce the presence of a gene without doing much laboratory work",[32] is at least an open question. There is no doubt that the success of the entire enterprise "The Human Genome Project" will very much depend on solutions taking into account the interests of all involved in a balanced way. HUGO should therefore continue its endeavours in seeking for such solutions.

Not surprisingly, the attitude of industry towards accessibility of raw data sequenced with their money is somewhat different. Companies like SmithKline Beecham, which invested US$125 million in The Institute for Genomic Research (TIGR) and Human Genome Sciences (HGS), did so to secure themselves privileged access to the sequenced data. Although the HGS-TIGR database is in large part accessible for researchers world-wide, it is so only under some restrictive terms. Those wishing to obtain access have to agree to notify the database sponsor of any patentable invention made with the data or clones received. Although the intellectual property rights remain with the academic institution and the institution is free to negotiate with third parties, it cannot offer terms less demanding than those it offered to the database owner with whom it has to negotiate on a case-by-case basis.[33] Consequently, HGS and SmithKline Beecham advocate that the existence in the public domain of the nucleic sequence of a portion of a gene, or an extended amino acid sequence of part of a protein, may constitute a level of "obviousness" or "anticipation" sufficient to deny

a patent to those who subsequently discover the full gene or the complete protein and elucidate function, although this may not apply in all cases. Therefore, according to their view, any academic or industrial group embarking on a campaign to place extensive human EST gene sequence data in the public domain would create a major obstacle to others seeking to patent the full-length gene and its function at a later date.[34] This view, however, is not shared by all pharmaceutical companies. Merck from the United States, for instance, strongly supports the free accessibility of raw sequence data, viewing the data as a genomic research tool which should be available to all scientists for research purposes. Merck invested considerable amounts of money to provide the National Human Research Center at the University of Washington in St. Louis with freely accessible databases. Moreover, Merck, in 1994, initiated the Merck Gene Index Project to generate a catalogue of cDNA clones of expressed human genes with associated ESTs that is publicly available to scientists in both the public and private sectors world-wide. The Merck Gene Index contains more than 245 000 EST sequences, representing over 91 Mb of DNA from about 150 000 clones.[35] A similar approach seems to be acceptable to pharmaceutical and chemical companies in respect to the accessibility of data which will be generated from sequencing pathogenic micro-organisms. A project is under examination according to which companies could jointly fund such sequencing work and enjoy for some limited time privileged access to the raw data, without those data being patented by the sequencing centres.

VI. CONCLUDING REMARKS

In retrospect, the treatment which the results of human genome research have enjoyed by patent law protection in the past seems adequate. No severe impediments to science and research have so far resulted from patents granted for full-length genomic and cDNA sequences with known functions. Patents in biological materials were instrumental for the emergence of a whole new biotechnology branch. Without patent protection a number of important diagnostics and therapeutics would barely have reached the markets by now. Although the dimension of the Human Genome Project puts the whole area into a new perspective, it does not as yet require fundamental changes of patent law, rather it requires its strict application. The public at large, and all others involved, will continue to benefit from the existence of intellectual property rights in the results of human genome research only if certain conditions are maintained:

- patent offices, by the strict application of all patentability requirements, should avoid allowing patents on speculative "inventions";

- the breadth of claims should be strictly commensurate with the contribution to the art, as described in the enabling disclosure provided in the specification;
- researchers should continue to enjoy a research exemption which is easy to apply.

At the same time the legislator is called on to monitor closely the impacts of product patents granted for genes, so as to react in time, if necessary, to prevent any untenable impediment to research and development in this crucial area of technology.

Speaking as a European patent lawyer, it is necessary to conclude that European patent law must close one substantial gap in order to put European scientists on equal footing with their colleagues in the United States: if the funding agencies impose and the public at large expect that all sequenced data be made immediately accessible via databases, they should also provide European researchers with a "grace period", which will allow data to be published without losing all possibilities for subsequent patent filing. This grace period should be included into the EPC as well as national patent laws. In order to have a harmonized situation world-wide and to be able to use the benefit of such a grace period internationally, it should precede the Paris Convention priority date.

NOTES

1. Co-authored by F.K. Beier, R.S. Crespi and J. Straus (1985), Paris.
2. *Ibid.,* No. 7 at p. 92.
3. See Thomas *et al.* (1996).
4. According to unpublished data collected by the former Office of Technology Assessment (OTA), United States Congress, however, the United States Patent and Trademark Office (US-PTO) alone issued 1 289 human DNA sequence patents from 1980 to the end of 1993.
5. See Rosenberg and Nelson (1994), Table 6 on p. 339.
6. According to Thomas *et al.* (1996). The unpublished OTA data differ somewhat, but also indicate that some 500 DNA sequence patents issued by the US-PTO were assigned to the US universities and the US Government.
7. Statement made by David Galas, then DOE HGP Director before the Panel on Gene Patenting of the OTA in 1993.
8. A minimum non-exclusive licensing fee of US$10 000 per annum and in case of exploitation of products obtained by using this technology royalties of between 0.5-10 per cent were due (*cf.* Feit, 1989).
9. For the US situation, reference is made to Roberts (1991). It should also be recalled that even Ministers for Research and Technology from France (Hubert Curien) and Italy (Antonio Ruberti) clearly opposed the NIH move and expressed their views in letters sent to Science magazine (Curien, 1991; Ruberti, 1991). The British Science Minister, Alan Howarth, on the other hand, defended the Medical Research Council (MRC) who, following the NIH example, had filed patent applications for ESTs, as a pragmatic decision, necessary to protect the interests of the UK economy. Howarth, however, at the same time acknowledged the long-term negative impacts of patenting ESTs on international co-operation and announced a waiver of all MRC actual or potential rights should an international agreement be reached not to patent ESTs (Howarth, 1992).
10. *Cf.* Science and Technology Committee (1995).
11. See Adler (1992) and Eisenberg (1992).
12. See Adler (1992).
13. See Murashige (1993).
14. *Cf.* Caskey *et al.,* "HUGO Statement on Patenting of DNA Sequences", Genome Digest, April 1995, pp. 6-9.
15. See Anderson (1994).

16. See Gugerell (1994).
17. See Stone (1995).
18. All quotations from the "CTNS Press Release, Patnt. PR Sept. 25, 1995".
19. See Cole-Turner (1995).
20. See Hudson *et al.* (1995).
21. See Brody (1989), also referring to Walters, Director of the Center of Bioethics of the Kenndey Institute of Georgetown University, Washington, DC. Along the same lines also Zimmerli (1994).
22. Official Journal of EPO (1995); see also Straus (1995).
23. Official Journal of the EC (1989).
24. COM(95)661 final.
25. For the sake of completeness, it should be added that on 29 July 1994, France amended Art. 7 of its Intellectual Property Code by Law No. 94/653 and declared unpatentable the human body, its parts and products and the knowledge of the entire or partial structure of the human gene, as such, ... as inventions, the publication or exploitation of which would be contrary to order public or morality. According to Galloux (1995), however, this new provision has not changed the former law, but rather restated the old principle allowing the patenting of products of nature, provided the patentability requirements are met.
26. Washington, DC, 1995.
27. See Roberts (1990).
28. See Cohen (1995).
29. See the Bermuda Statement (1996).
30. See Marshall and Pennisi (1996).
31. See Khan (1996).
32. See Shaffer (1996).
33. See Marshall (1994).
34. See Poste (1995).
35. See Merck Gene Index Update (1996).

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A PERSPECTIVE ON CROP BIOTECHNOLOGY FOR THE FIRST HALF OF THE 21st CENTURY

TABLE OF CONTENTS

This article was written by Dr. Willy de Greef. Dr. de Greef is Technology Assessment Manager of the Flanders Interuniversity Institute of Biotechnology (VIB) in Belgium. He has a professional background in crop biotechnology, tropical crop research management and technology transfer to Africa.

I. INTRODUCTION AND DEFINITIONS

This article is not about crop biotechnology, at least not in the sense of a technical assessment of the supply of scientific progress and technical opportunities in the field. The objective is, instead, to attempt a reflection from the demand side of primary production of the planet, and to review briefly if, how, when and how much crop biotechnology can contribute to supply the goods needed to feed, clothe, house, warm, heal and entertain the 9 to 11 billion people expected to live on the Earth by the year 2050. Before going into the argument, we will, as is usual when discussing this nebulous (some would say tentacular) field of enterprise, define what we mean, for the purpose of this article, by biotechnology.

Since the publication of the Biodiversity Convention, commentators of biotechnology have a free licence to define the field in almost any way they want. The Biodiversity Convention resolved the dilemma of definition of the subject area by the simple device of offering two definitions, one very narrow (essentially rDNA technology), the other all-inclusive (essentially including all the applied life sciences). We will use the more or less flexible label of "modern cell and molecular biology" to define the subject area. This would include:

- all rDNA technology applications (including transgenics, molecular markers) which can be summarised under the heading "advanced genetics and breeding";
- all applications of cell and tissue culture technology (including cloning and *in vitro* multiplication, haploid cycle breeding, *in vitro* mutagenesis and selection);
- "advanced agronomy" as exemplified by the development of sophisticated biopesticides and biofertilizers; and
- biochemistry and its direct applications in food production.

We will interpret "agriculture" as "food production". This will enable us to discuss a number of developments aimed at improving post-harvest treatment of agricultural products.

II. THE "DEMAND" CHALLENGE

Perceptions and opinions as to how agriculture should evolve over the next half century differ widely. Most observers would agree, however, that the following expectations have to be met convincingly by any food production system that hopes to provide a more or less permanent solution to the world's primary production needs:

- it has to be able to feed 9-11 billion people;
- it has to do so on less land area than at present;
- it has to be sustainable; and
- it has to be able to do so at an acceptable environmental and socio-economic cost.

Recent prognoses on world population in 2050 range from 9 to 11 billion people. Almost all of the increase (from the current 5.7 billion) will occur in what are now developing countries.

Land availability is almost impossible to estimate 50 or more years in advance. Doom scenarios on world agriculture and environment tend to show dramatic decreases in arable land, but these often reflect extrapolation from past, clearly unsustainable, trends. Nevertheless, two major problems stand out as likely to increase dramatically over the coming half century: land degradation through improper use; and availability of water for irrigated agriculture. To this can be added the uncertainties generated by the generally expected climatic changes.

Policy strategies for the long-term future tend to include wide-ranging assumptions (or expectations) on the capacity of the agricultural system to provide not only the current range of products, but to expand the range to the supply of energy and a much increased supply of raw materials. Apart from food and beverages, feed and fibre (clothing and paper), we count on agriculture for the supply of raw materials (rubber, fatty acids, industrial carbohydrates, etc.), building materials (wood, bamboo, etc.), energy supply (wood, oils, ethanol, etc.), entertainment (ornamentals, parks, etc.), bioremediation, etc.

This article will cover the problem of food security with the help of crop biotechnology at the global level, because the food market is global, and because the effects of crop biotechnology on the distribution of crop production will affect global streams of agricultural products. Priority setting at the interface between the agricultural system and society is radically different in developed *vs.* developing countries.

Developed countries have:

- a more or less stable population;
- structural overproduction of agricultural products; and

– emphasis on the environmental impact of agriculture.

Developing countries have:

– a rapidly increasing population;
– a developing structural food deficit; and
– emphasis on production and development over environmental considerations.

Crop biotechnology can contribute to each of these challenges. It is the task of policy development to ensure that this is done in a rational way.

III. FROM TECHNOLOGICAL TO ECONOMIC REALITY

Biotechnology is a new and powerful toolbox. It does not in itself constitute or deliver end-products, but rather changes production processes and expands the range of products that can be made. This has important implications for the way in which crop biotechnology is integrated into the economic fabric.

Especially in crop biotechnology, the transformation of biotechnology from technological wizardry into economic reality requires a culture shock from the socio-economic agents that will make it happen. However, these same agents play a vital role in the process, since they have the complementary skills, technical, business and legal, to carry the intermediate products generated by the biotech industry to the final customer: the farmer and/or the consumer.

In the course of this article, we will only briefly speculate on new, as yet unproved, technologies that may or may not revolutionise the agricultural sector over the next half century. Since speculation it is, it would seem more productive to concentrate on the opportunities offered by the technologies discovered over the past few decades, and to the process of their incorporation into agricultural practice.

Crop biotechnology is now in the lag phase of a classical new technology revolution. Such revolutions usually consist of three clearly distinguishable steps:

1. Early scientific and/or technological breakthroughs:
   – These create inflated expectations, especially about time-frames for converting scientific breakthroughs into technical realities, and later into marketable products.
2. Technology development and consolidation, product development:
   – During this stage, which almost always takes considerably longer than outsiders expect, disappointment with the technology sets in. Some technologies even die in this stage if they also lose the long-term financial support to carry them through. The non-technological aspects of

product and market development can add to the delays in economic development.

3. Market introduction and consolidation:
   - At this stage the real revolution takes place, although it will often go relatively unnoticed.

For medical applications, we are currently witnessing the start of the third stage of the process. In the case of agricultural biotechnology, we are coming to the end of stage two. Because of its diffuse impact, not only on the possibility for new product development, but perhaps even more on the way in which agricultural input products will be developed in the future, agricultural biotechnology has already caused a revolution in the way agribusiness is structured, and in the approaches to further development.

To assess the challenge facing biotechnology in the agricultural sector in the 21st century, it is useful to look at present production trends, and ask whether, and under what conditions, these can be sustained, and if they are sustainable with present technologies in the first place. During the past decades, food production has outstripped population growth, due mainly to yield improvements. Most of these are the result of increasing inputs in agricultural systems: water, fertilizers, phytochemicals, etc.

Assuming an increase of the world population by the year 2050 to 9-11 billion, the gross availability of food has to increase by at least 50 per cent: 1 billion

Table 1. **Evolution of crop production and population**

	1970	1975	1980	1985	1990	% change
Population (billions)	3.596	3.951	4.450	4.837	5.300	+47
Cereals						
Area (million ha)	693	732	718	721	705	+2
Prod. (billion tonnes)	1.231	1.362	1.588	1.847	1.971	+60
Yield (tonnes/ha)	1.78	1.86	2.21	2.56	2.76	+55
Legumes						
Area (million ha)	101.4	113.9	111.7	117.9	124.8	+23
Prod. (million tonnes)	92	114	127	151	170	+81
Yield (tonnes/ha)	0.91	1.00	1.14	1.28	1.34	+47
Tubers						
Area (million ha)	51.2	51.7	47.7	46.0	42.1	−18
Prod. (million tonnes)	543	553	560	586	544	+0
Yield (tonnes/ha)	10.6	10.7	11.8	12.7	12.9	+22

Source: FAO, *Production Yearbook* (various issues).

tonnes more cereals, and almost 100 million tonnes more legumes, per year. However complex the problem of world nutrition may be, the basic data are surprisingly simple. The bulk of the physically available food and feed production world-wide is determined by only three factors: area planted, yield and post-harvest losses, in the following relationship (where F = food; A = area; Y = yield; and L = post-harvest losses):

$$F = A \times Y \times (1 - L)$$

Although FAO estimates point to a large area of presently unused arable land, it would be undesirable to bring this into production, because in the future, as is the case today, habitat destruction is likely to be the prime cause of environmental degradation. Moreover, parts of the currently cultivated land area are so highly degraded that they should be considered lost. Large areas in the tropics are still cultivated on shifting cultivation cycles, requiring a multiple of the area actually cultivated in any given year. Finally, dryland agriculture in parts of the tropics is only made possible by harvesting water from areas much larger than the cultivated field surface.

A comment on the set-aside policies of some production countries: there is a tendency, especially in Europe, to take out of production, at least temporarily, some of the most productive land. While this may make good sense in the context of a policy for surplus reduction on a regional basis, it is environmentally destructive. On a global scale there is no surplus production. Therefore, reductions on these highly productive soils have to be compensated by increases in other places, requiring much larger areas.

Post-harvest losses have two main causes: storage and distribution losses, many of them biological, and processing losses. The food industry has been spectacularly successful in using by-products of crops, thereby gaining maximum results from the primary yield. Some of the most conspicuous successes have been controversial, and one of the criticisms that have been laid at the door of the biotechnology industry is that, by making it possible to "reconstitute" many types of food from the cheapest available sources, it has effectively destroyed the markets for traditional producers (*e.g.* high fructose corn syrup, cocoa butter substitutes). By expanding the range of possible uses for base products, biotechnology applied to reduction of processing losses is one of the major tools for assuring future food availability. Many sources of storage loss are directly related to the physiology of the crop (*e.g.* softening of stored tomatoes) or to pests and diseases. Therefore, most of the genetic approaches used on similar problems with standing crops in the field can also be applied here.

The bulk of the increased production will have to come from yield increases of between 40-50 per cent. This is a global average, hiding large regional differences. Over the past few decades, while overall crop productivity has increased

significantly in all the food-producing regions of the world, it has outstripped population increase in some (Europe, North America, Asia), and lagged behind population growth in others (most notably Africa). As a result, some of the regions of the world with the fastest population growth are rapidly becoming large net importers of food. While most analyses agree that on a global scale we will be able to make up these shortfalls, it is at the cost of an increasing dependence of regions such as Africa on external food help; which is considered by many to be unacceptable. Therefore, if self-sufficiency were imposed as a requirement, food production in such regions would have to grow by much more than 50 per cent over the next half century. On the other hand, since the same constraints on arable land apply in these regions (most notably Africa and South Asia), yield increases will have to be much more dramatic than in the rest of the world. Fortunately, these regions at present have the lowest yields of the world and it can be expected that agricultural and genetic improvements will deliver the required growth, provided they are properly applied. This requires an integrated approach to agricultural R&D and extension, in which biotechnology will play a prominent role.

Generally, technology should be applied to the existing problems by a strategy of "nudging" on the basis of a number of "desirable" principles. Any improvement, by whatever technology, that delivers :

- substitution of genes for chemical or physical inputs;
- increased output/input ratio (for land, minerals, chemicals, energy, water);
- decreased need for agricultural production on marginal land;
- reduction of barriers to technology access for farmers;

would be a good candidate for introduction into cultivation systems. High quality seed material fits these requirements much better than almost any resource. High quality seed has several features:

- it is genetically superior;
- it is free of disease;
- it is physiologically in optimal condition; and
- it is available at reasonable cost.

From these premises, it is possible to set a programme for the contribution of biotechnology to agricultural development. Although it is impossible to estimate how far the technology itself will develop in the next few decades, on the basis of present trends it can be assumed that:

- Most pest and disease problems will be amenable to a durable solution, although this will probably happen by a dynamic interaction between pests/diseases and control measures. There is a widespread controversy on the sustainability of present strategies for developing pest and disease resis-

tance in crops through genetic engineering. This is essentially based on a misunderstanding of the nature of the interactions in an agricultural ecosystem. There is no such thing as a permanent solution to a pest or disease problem. The question is never if a disease will evolve a way around a control measure (be it genetic or chemical or cultural) but rather when it will do so. This expensive lesson was learned with antibiotics in human health care. By declaring the war on bacterial diseases over after the first campaign in the 1970s, the medical community only showed its lack of understanding of evolutionary biology. However, this early mistake does not invalidate the approach. The medical community has since learned what an evolutionary "arms race" is and, with the advent of molecular biology and biotechnology, is rapidly building up new and formidable arsenals of control measures. This implies a strategy of continuous monitoring and proactive development of new measures against pathogens. For agricultural biotechnology, this means that in 50 years, biotechnologists will almost certainly still be developing new batteries of pest- and disease-resistant genes. This is not a new strategy for crop genetic improvement; the same strategy has been used, through traditional breeding, for the control of fungal diseases in cereals, with significant success.

- Physiological limitations to growth and development will be at least marginally improved. The most advertised approach to this (genetic engineering of drought, cold, heat ... tolerance) is probably not the one that will be most widely used in practice, at least not in the short to medium term. As molecular markers become more widely available it is likely that these will assist in making rapid progress. However, it is unlikely that this work will lead to spectacular progress. The physiological barriers set by the basic needs of plants are already tested by some of the best varieties available today.

Three main technologies can be applied to achieve one of the above objectives. Although attention has focused on genetic engineering, it is not likely to have the heaviest impact, at least not in the short to medium term. However, on a 50 year time scale, it will. Within the next 20 years, *in vitro* culture and marker-assisted breeding will offer more straightforward approaches, especially for tropical crops.

Tissue culture technology remains a cheap, widely available technology for the creation and rapid multiplication of disease-free planting material, especially for clonally multiplied crops. One of the reasons why production of tuber crops in the tropics is in relative decline compared to that of cereals, is that it is much easier to deliver clean and healthy seed material for the latter. This need not be the case: it has been demonstrated in a number of developing countries that *in vitro* culture is a technology well suited for this type of environment, and some

of the best examples of successful technology transfer are to be found in this area.

Marker-assisted breeding is an application of DNA technology that has quietly caused an even more rapid revolution in crop development than genetic engineering. The spectacular fall of its cost, and the fact that the usefulness of the technique is immediately clear to any breeder of any crop, is leading to a massive effort to develop molecular maps for most crops of any agricultural significance. By reducing the number of combinations that have to be tested in the field, and the time to fix traits, it makes the power of very large breeding programmes available to crops which could not pay for them at the present time. This will contribute more than any policy to the conservation and possible reintroduction of a number of crops that are now under pressure of losing out to the major commodity species.

Both tissue culture and marker-assisted breeding will have an enormous impact on the development of tree crops. Trees have a number of inherent traits that would make them in principle a much more attractive proposition for sustainable high yield agriculture than annual crops. They require less capital to develop, are much more friendly on soils, have better resistance to sub-optimal growth conditions, and offer stability for agricultural communities. Some tree crops are spectacularly successful: coffee, cocoa, tea, citrus, oil palm and coconuts, rubber. Each of these crops forms the basis for successful economies. They also represent one of the most environmentally friendly ways to build up agricultural capital.

The main weakness of tree crops lies in the time it takes to achieve improvement. Long life cycles, combined with high initial establishment costs, combine to produce excessively long lag periods between genetic improvement and its introduction in agricultural practice for these crops. The possibility of dramatically reducing variety development costs by a judicious mix of marker use and clonal multiplication is only starting to be included in strategic planning for these crops. This will be even more important for the new tree crops presently under study.

IV. BUILDING ENABLING CAPACITY

There is no doubt that the three main current applications of modern biotechnology: tissue culture, marker-assisted breeding and genetic engineering, will have a profound impact on crop development in the next generation. How fast they are introduced into standard agricultural systems, however, is not in the first place a matter of technology development. Tissue culture is already a mature technology and, within a decade, the two other categories will also have matured. What is required, then, is a strategy for systematic exploration of the possibilities

offered by these technologies for the whole range of crops likely to be cultivated in 30 years or so.

There has been widespread fear in the early 1990s that biotechnology would increase the gap between major crops in industrial countries and those more important to developing countries. This perception was based on the analogy of some other areas of high-tech development (*e.g.* pharmaceuticals, nuclear energy) where entry barriers are enormous and growing. At present, it seems more likely that biotechnology, which is inherently low-cost, will follow the path of telecommunications and information technology, providing unexpected opportunities for leap-frogging for late entrants. Individual projects become far less risky, and cheaper, as more trained staff become available, and an efficient supply industry offers standardised equipment at decreasing prices.

In order to use these observations for the establishment of practical strategies, it will be necessary to build capacity in the following sectors, in addition to the technology itself:

- investment policy;
- human resources development;
- institution building;
- business development; and
- intellectual property rights.

Investment policies in agriculture must include a long-term vision of R&D. This means, in the first place, a strengthening of the capacity of existing plant breeding efforts. One of the most dramatic negative effects of biotechnology over the past decade has been that the lure and glamour of high technology has detracted attention and funding from traditional breeding. Since no application of biotechnology can reach the farmer without the intermediate step of an effective breeding system, it is imperative that this is reflected in policy. Recent developments in industrialised countries have shown that effective use of biotechnology in crops is achieved better in crop breeding centres, as part of a wider approach to agricultural problems, than in stand-alone biotechnology centres. This is a reflection of the nature of molecular biology and biotechnology as a powerful toolbox, able to speed up the development of end-products, rather than as a set of products by itself. The shift of centres of excellence of biotechnology research to agricultural research centres is a symptom of the maturation process of the technology.

ANIMAL BIOTECHNOLOGY

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This article was wiritten by Louis-Marie Houdebine. Professor Houdebine is Directeur de Recherches at the Institut National de la Recherche Agronomique. He studies milk protein gene expression and is involved in projects aiming at producing recombinant proteins of pharmaceutical interest in milk, generating transgenic animal models for biomedical studies and improving animal production by gene transfer. Professor Houdebine is a member of two French biosafety commissions: the Commission de Génie Génétique and the Intercommission II of the Institut National de la Santé et de la Recherche Médicale.

SUMMARY

Genetic engineering applications relating to farm animals are developing more slowly than had been surmised 15 years ago. Yet real progress has been achieved. Genome mapping is being used to draw up new breeding programmes and to identify hitherto unknown genes. Gene transfer is still considered to offer much scope for achieving faster breeding or for taking advantage of mutations that nature can only produce at a much-too-low probability. Reproduction techniques are becing mastered (*in vitro* production of embryos, cloning, chimaera production, etc.), and as a result the genomes of useful animals can be spread more rapidly and gene transfer facilitated.

The production of recombinant proteins for pharmaceutical purposes using transgenic animals is becoming an industrial reality. The same techniques can be used to optimise the composition of milk for human and animal consumption. Disease control is starting to receive the impact of genetic engineering techniques (preparation of recombinant protein vaccines, use of viral vectors for vaccination, use of naked DNA for vaccination, genetic vaccination through transgenesis). Some transgenic domestic animals are used as models for biochemical studies. The prospect of transgenic pigs constituting a source of organs for humans is coming very close. All these techniques are variously perceived by public opinion. Reservations concerning the application of biotechnology are chiefly nurtured by ignorance of the fundamental laws governing living organisms and of the exact nature of the challenges facing biotechnology. These new techniques have developed so rapidly that a gap has formed between reality and public perception. The application of recent discoveries in biology raises new specific problems of biosafety and bioethics. It is essential that public opinion have a better perception of reality if the best options for mankind are to be taken under satisfactory conditions.

I. INTRODUCTION

The routine use of genetic engineering techniques which began 20 years ago has sent a succession of shockwaves through the research world, industry and

public opinion. The possibility of identifying, modifying and using practically any gene belonging to a living organism has opened unprecedented prospects for mankind. When the news of the first transgenic mice to be produced came out just over 15 years ago, it was greeted with overenthusiasm by some, panic by others. So far, fundamental research has been the main beneficiary of all these techniques. The use of micro-organisms, plants and animals has been significantly modified by the contributions of genetic engineering, but advances have clearly been made more slowly than expected, especially as far as animals are concerned. This is due to the basic complexity of living organisms, the large investment required and the extreme reservations of public opinion. After an initial period of enthusiasm which then petered out owing to the limited success achieved, we are now entering a phase where animal biotechnology can develop along more predictable lines. Success is largely governed by the size of investment made, but also by the level of public acceptance of biotechnology. The gap that has widened between scientific and industrial reality on the one hand, and public perception on the other, must definitely be bridged in order for more enlightened choices to be made. This is especially true in the animal field, which is of closer concern to mankind.

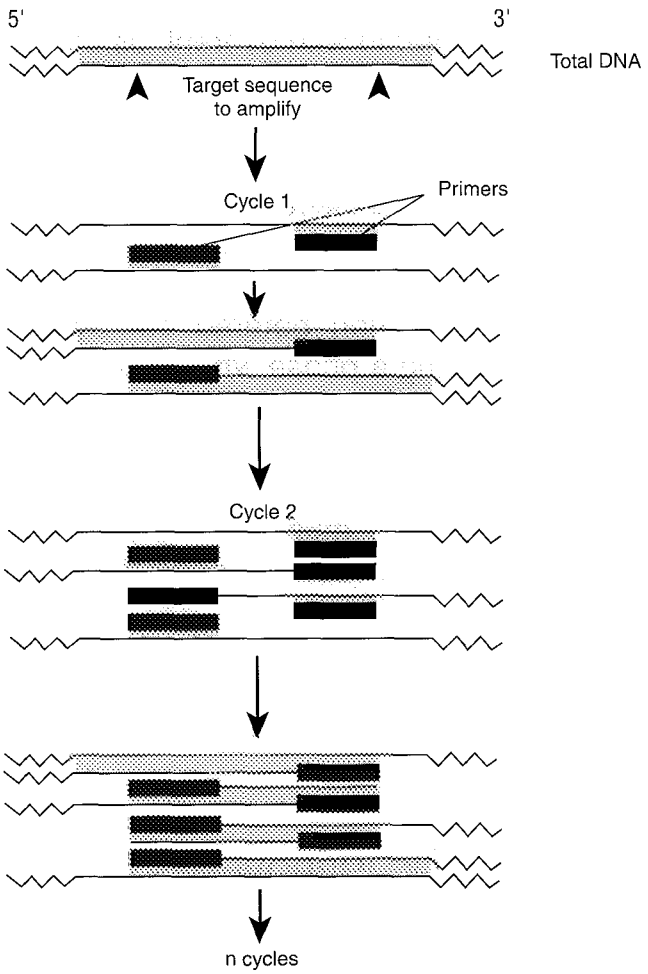
The purpose of this article is to summarise the current status of research and applications in animal biotechnology and to identify the factors hindering their development.

II. GENETIC IMPROVEMENT OF ANIMALS

Ever since the laws governing the transmission of hereditary traits were discovered, geneticists have learned how to select animals. Achievements in this field are widely documented. Increasingly sophisticated breeding programmes can still lead to very substantial productivity gains in domestic animals. Until very recently, these methods did not entail any precise identification of the genes concerned. Traditional genetic selection consists in spotting as precisely as possible the effects of spontaneous mutations and then breeding animals showing the best results. This approach therefore depends on the vagaries of nature since mutations may or may not be of interest. The effectiveness of the selection also depends on pertinent observation of the animals.

Precise knowledge of specific DNA regions enables finer genetic selection. Ruminant and pig embryos can, for instance, be sexed within just a few hours. A few cells are taken from the embryo by microsurgery. The specific region on chromosome Y (which is present in males only) can be amplified by a factor of one million within two hours through the gene amplification technique (PCR) (see Figure 1) (Cotinot, 1992). In theory, this technical procedure can be applied to any

Figure 1. **Identification of a DNA fragment through gene amplification (PCR)**



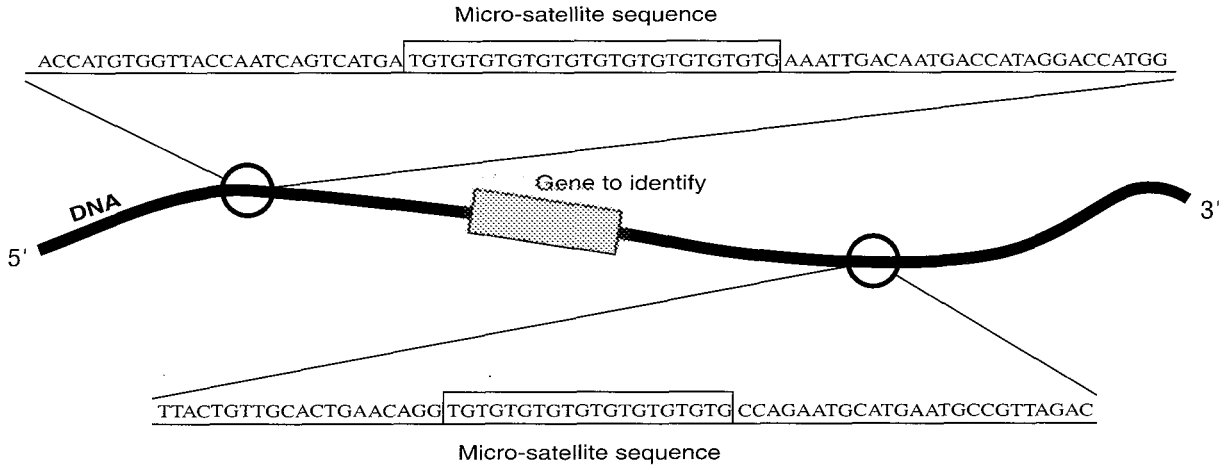
Note: Oligonucleotides (approximately 20 nucleotides) corresponding to DNA sequences surrounding the region to be amplified are used by a DNA polymerase which merely exponentially synthesises the region concerned.
Source: Author.

gene fragment: many genes can therefore be analysed from the same samples. In newborn or adult subjects, various tissues (blood, skin fragments, etc.) can be used for such analyses. *In vitro* multiplication of cells sampled from embryos is fully feasible, so that a large number of genes can be analysed from just a few cells (in theory at least one cell). The PCR technique and a few others very soon enabled the most useful genes in milk proteins to be defined. The selection of breeding bulls has become markedly faster and more efficient.

More generally, genome mapping offers the prospect of selection based on the identification of specific DNA sequences. Plant and animal genomes contain large numbers of repeated sequences of various sizes, referred to as mini- and macro-satellites. These sequences are distributed throughout the genomes. They are transmitted from one individual to the next, but with a much lower conservation level than functional genes. One of the purposes of genome mapping is to locate the satellites. With the PCR technique a designated micro-satellite can be specifically amplified. Systematic analysis covering an adequate number of animals can establish correlations between the presence of easily identifiable micro-satellites and a specific genetic trait of interest (Figure 2). A breeding programme can therefore be based on straightforward, rapid and reliable analysis of micro-satellites. Thanks to this new approach genetic selection is already becoming more successful and also more precise. The less global nature of this type of selection will reduce the level of in-breeding which unavoidably arises from genetic selection.

Correlation between a given genetic trait and the presence of a micro-satellite leads to precise identification of the gene or genes concerned, which can then be isolated. Gene identification makes selection even more accurate (as in the case of milk protein genes), improves knowledge of the role of genes in the life of the

Figure 2. **Identification of a region of interest in a genome by means of micro-satellites**



Note: Specific amplification using PCR (see Figure 1) of two micro-satellites (TG)n of different lengths can be done by means of primers either side of each micro-satellite. By correlating micro-satellites and a genetic trait of interest, animals can be selected on the basis of the presence of the micro-satellites and the genes required can be progressively identified.
Source: Author.

organism and, where applicable, allows their transfer to other animals in order to transmit the mutation concerned.

Pig and bovine genome maps are already being exploited. Those of sheep, goats, chicken, trout, etc., are being drawn up. The list is growing more and more rapidly inasmuch as mapping techniques are advancing, but also because there are similarities in the genomes of the various animal species.

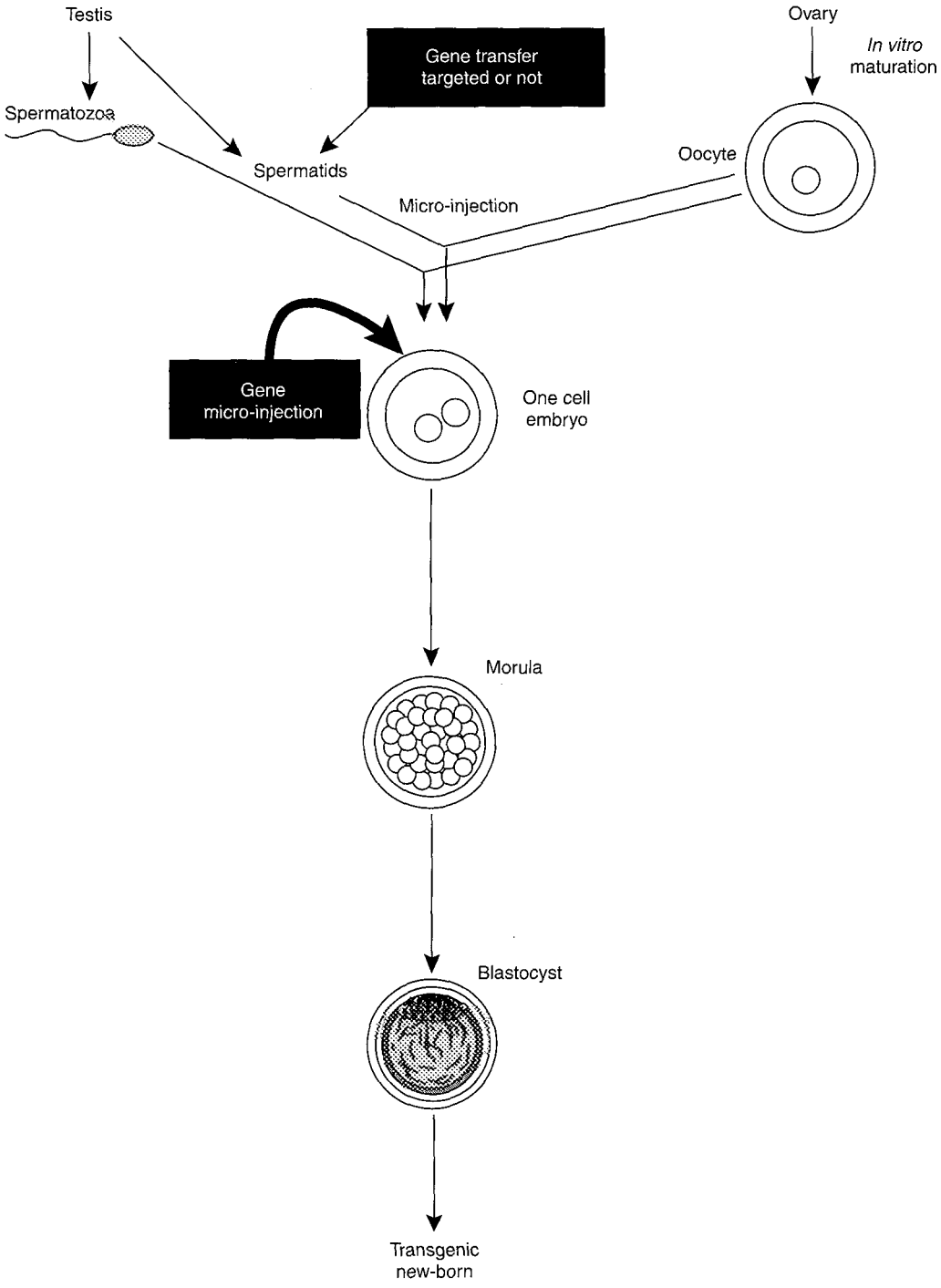
III. ANIMAL REPRODUCTION AND TRANSGENESIS

Through expert reproduction techniques, herd productivity has markedly improved. Artificial insemination is now used for most domestic animals. It is routine practice to collect embryos after super-ovulation, free them and transfer them to recipient females. Both these techniques have been instrumental in speeding up genetic selection. It is possible to collect egg cells from slaughter-house ovaries or directly from live animals endoscopically at each sex cycle. *In vitro* ova maturation and fertilisation multiply the number of germ cells that a female can produce. Through such techniques embryos can be made available in large quantities for researchers wishing to investigate early development or to micro-inject genes into ruminants. Embryos can also be grown *in vitro* up to the blastocyst stage. In this way, non-viable embryos can be spontaneously discarded and viable ones can simply be reintroduced into the uterus endoscopically instead of into the Fallopian tubes by conventional surgery. These techniques are now available for bovine animals, sheep and goats (Crozet, 1992).

Spermatids, which are the immediate precursors of spermatozoa, can be used for *in vitro* fertilisation by means of direct micro-injection into the oocyte. By culturing spermatocytes until they are fully differentiated into spermatids, relatively large numbers of male cells could be obtained and genes transferred, whether targeted or not. Under these conditions, spermatids could act as cell vectors for foreign genes (Figure 3).

Chimaera can be obtained by introducing early embryo cells into an embryo having reached the same stage. The exogenous cells blend with those of the host embryo and may even participate in the formation of germ cells in adults. Chimaera can be obtained in mice, rabbits, ruminants, pigs, chicken and certain fish. In the case of mice and, apparently, chicken, embryo cells from the internal cell mass of blastocysts or primary germ cells can be cultured for long periods of time to form stable lines of ES and EG cells, respectively. These so-called totipotent cells can participate in the formation of chimaera. While they are being cultured, the cells can receive foreign genes through conventional transfection or a process of homologous recombination. Under such conditions a mutated or non-mutated exogenous gene very precisely replaces the endogenous homologous gene. The

Figure 3. **Use of embryos at the single cell stage obtained from ova matured *in vitro* and after *in vitro* fertilisation**



Note: This can be facilitated by micro-injection of the sperm or spermatid. A gene can be micro-injected into one of the embryo's pronuclei at the single cell stage.
Source: Author.

generation of whole organisms from totipotent cells via chimaera therefore amounts to mutating a specific gene in the whole organism in a highly precise and specific manner (Figure 4).

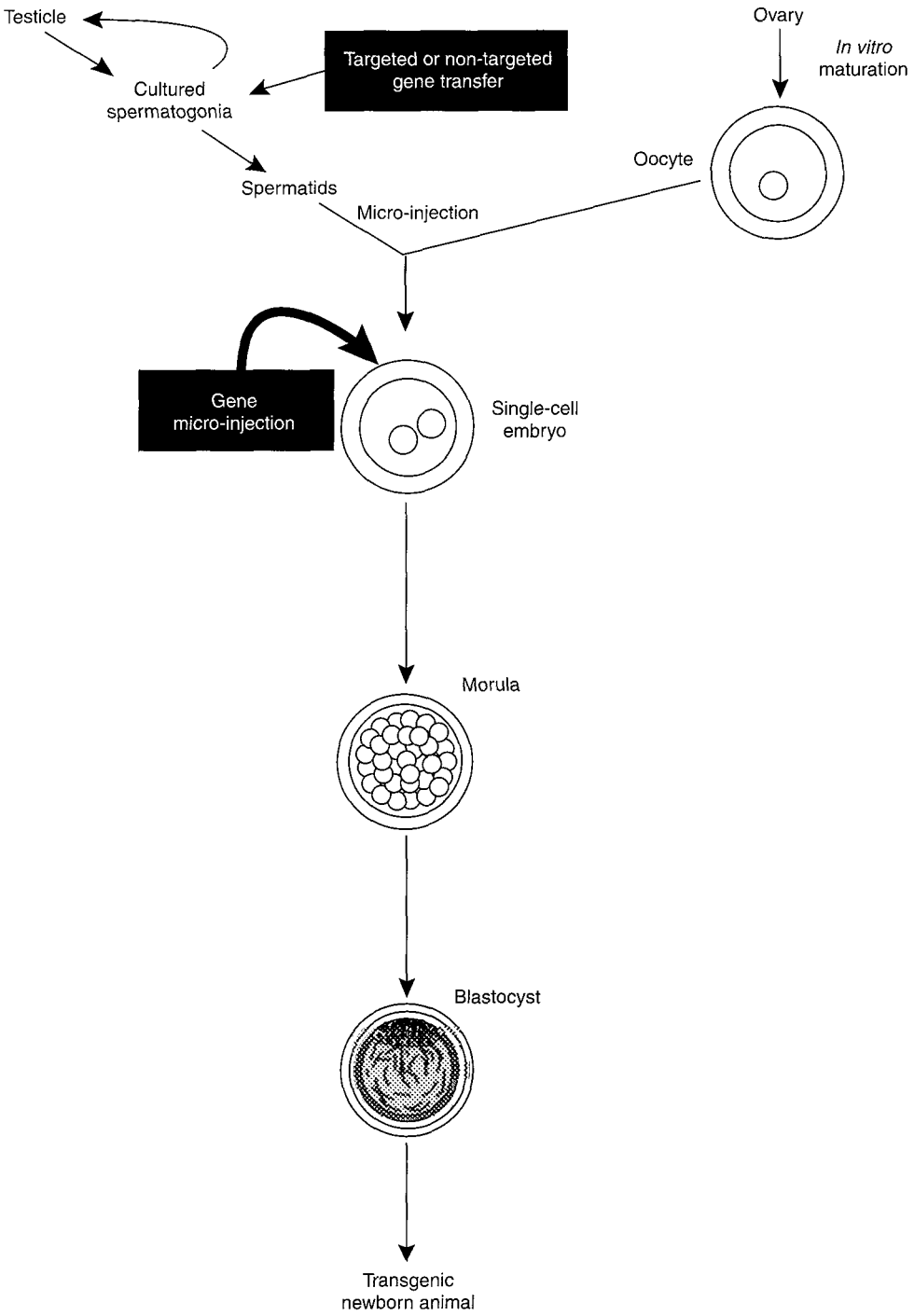
Unlike plants, animals cannot be cloned from somatic cells. Cloning can be done, however, provided a relatively limiting protocol is followed. This consists in: *i)* removing the genetic material of an oocyte by microsurgery to obtain an empty cytoplasm; *ii)* isolating cells from early embryos (blastomeres); *iii)* introducing a blastomere between the pellucid zone and the embryo's plasmic membrane; and *iv)* merging the membranes of the ovum and the blastomere and activating the new embryo created by means of an electric shock (Figure 5). This technical procedure can be successfully followed in rabbits and ruminants. One to seven individuals (on average two to three) can be obtained from a single embryo (Renard and Heyman, 1992). This figure is too low for stockbreeding purposes. At least ten identical embryos would be required for the technique to lead to any significant genetic improvement. There is reason to believe that these techniques can be improved and it will even be possible to clone embryos from other embryos obtained by cloning. One single embryo could therefore theoretically produce several hundred identical animals. Stock production would therefore increase correspondingly. The use of identical animals produced in this way would have to be strictly controlled to prevent rapid and highly damaging loss of genetic diversity (Colleau, 1993).

A recent study has shown that live lambs can be obtained from sheep embryo cells cultured for several weeks then transferred into the cytoplasm of enucleated oocytes (Figure 5). Such cells, which are regarded as multipotent and no longer totipotent, cannot participate in the formation of chimaera but they can be reprogrammed using the ovum cytoplasm to resume a full growth cycle (Campbell *et al.*, 1996). The yield is still very low in sheep. There has been only partial success in cows because gestation is prematurely shortened when this technique is used. However, this can undoubtedly be perfected. The outlook is highly promising since such techniques could be extended to other domestic species and in theory, it is possible to obtain animals that have received a foreign gene through conventional transfection or through homologous recombination targeting.

Gene transfer is therefore possible in mammals and the lower vertebrates through straightforward micro-injection into pronuclei or the cytoplasm, respectively. In birds, the use of retroviral vectors is possible, albeit with a low rate of success. The use of totipotent cell strains to create chimaera is not possible except in mice, whereas multipotent cells can be used if cloning is carried out.

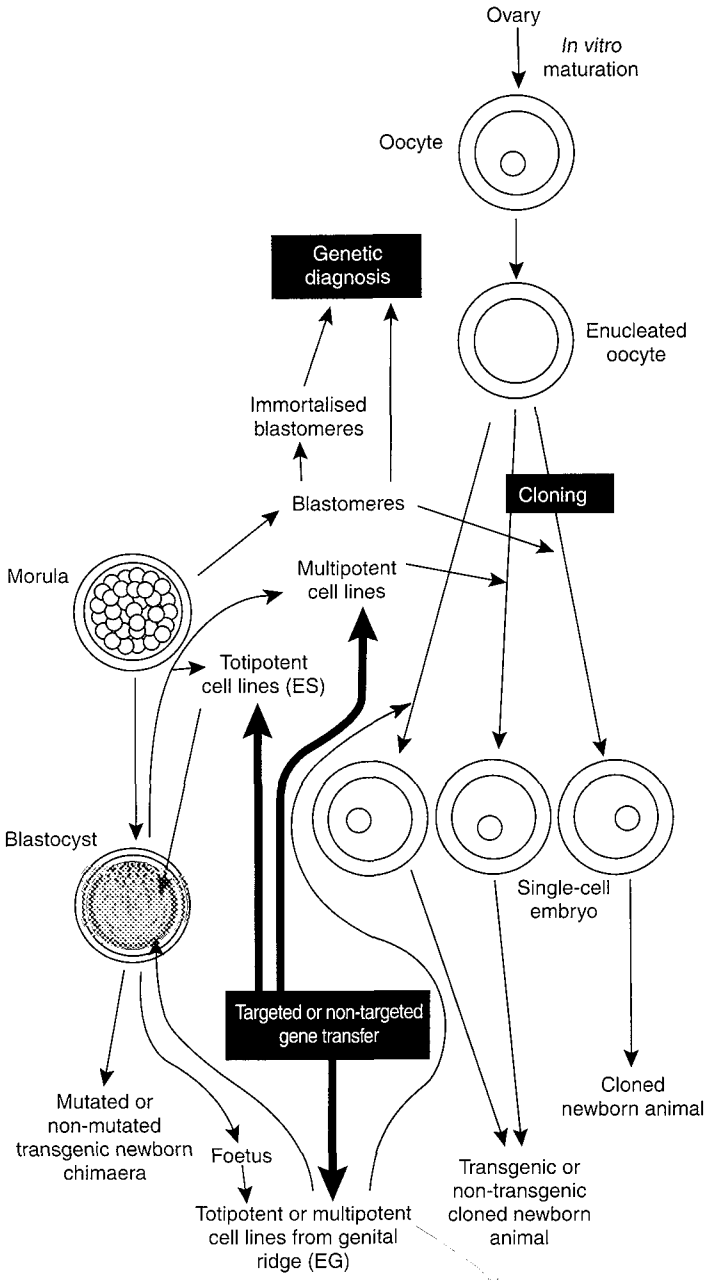
Entire genomes can be manipulated in lower vertebrates and invertebrates. By applying thermal shocks or high pressure to the embryos of certain fish, and especially the salmon family, the first cell division can be disrupted. The embryo

Figure 4. **Use of spermatozoa obtained by *in vitro* culturing to fertilise ovocytes**



Note: In theory it is possible to bring spermatozoa precursors to maturation *in vitro*. During culture, genes could be transferred to the male germ cell using a traditional or targeted method (through homologous recombination).
Source: Author.

Figure 5. **Use of totipotent and multipotent embryo cells to transfer genes**



Note: Parent embryo cells from early embryos (ES cells) or the foetal genital ridge (EG cells) can be cultured and reintroduced in early embryos so as to participate in their development. Genes can be transferred to the cells, whether targeted or not, during culturing. Embryos can be cloned by transferring the nucleus of early embryo cells into the cytoplasm of enucleated ova. Multipotent cells cultured for several weeks can also provide nuclei for embryo cloning. In theory, genes can be transferred during culturing.

Source: Author.

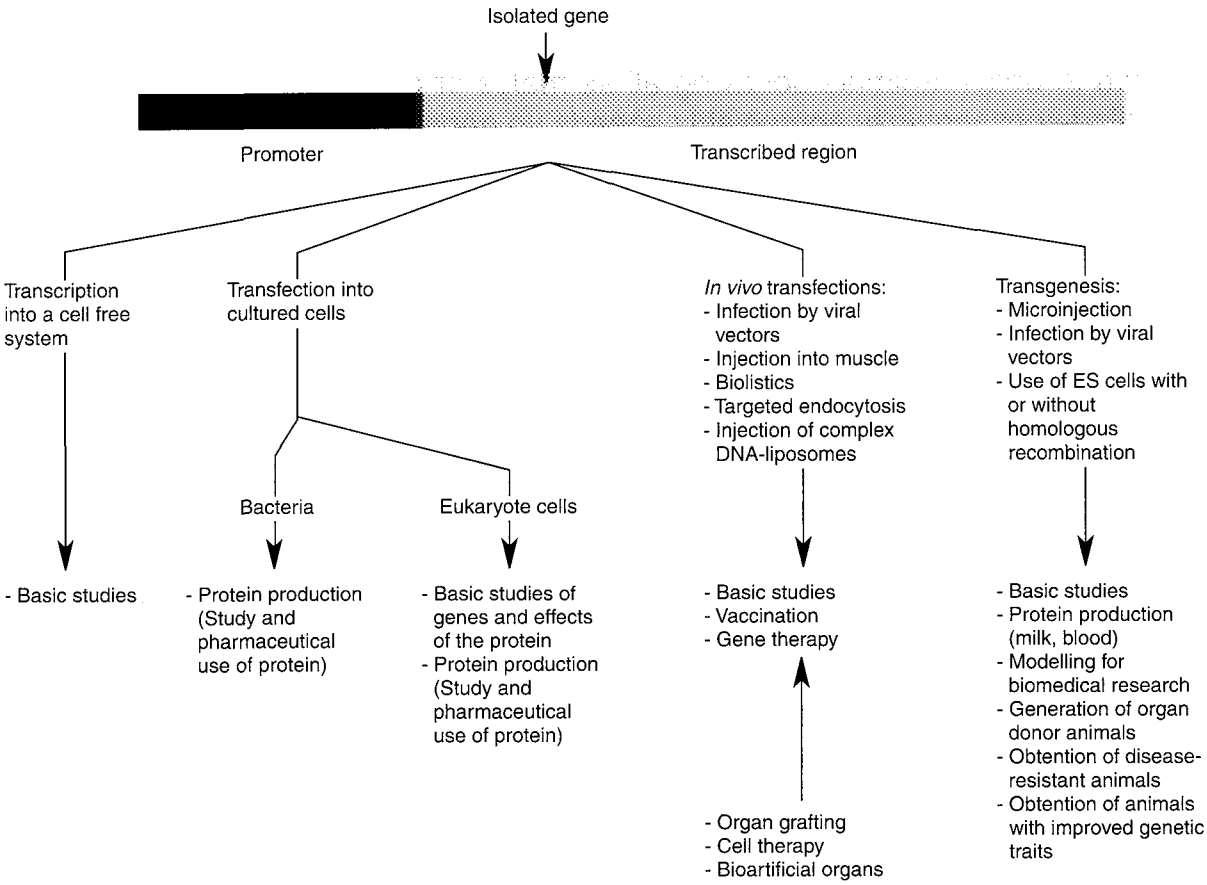
ends up with a four-fold stock of chromosomes (and not two-fold). Such treatment does not prevent normal growth, and tetrapoloid animals can be obtained which are sterile. This is an advantage in fish farming, inasmuch as growth is not interrupted during the normal reproduction period. Various procedures can also be used to obtain animals behaving all as males or all as females. The ensuing controlled consanguinity appreciably speeds up genetic improvement (Chourrout, 1988).

IV. PRODUCTION AND USE OF RECOMBINANT PROTEINS

A recombinant protein is a protein produced by an organism other than that of origin following gene transfer. Insulin, growth hormone or erythropoietin produced by bacteria or animal cells are recombinant proteins now routinely used for human therapeutic purposes. Protein from blood, but above all from the milk of transgenic animals, is now produced on an industrial scale. Several dozen proteins have been created by combining the promoter of one of the milk protein genes with the coding region of corresponding genes (Houdebine, 1994). Clinical tests are about to be conducted on three of these proteins. It is expected that hundreds of proteins, and especially monoclonal antibodies, which will have many different therapeutic and diagnostic uses, will be prepared by means of this process, which presents several advantages. The proteins are often well-formed and functional. Their production is about 50 times cheaper than when animal cell fermenters are used. The system is also relatively flexible, since production can be increased by simply multiplying the animals. This is an important process for the pharmaceutical industry, but it will only have a negligible impact on stock-breeding since, at best, only a few animals will be concerned.

Milk accounts for 30 per cent of protein consumption in affluent countries. The composition of milk for human consumption can conceivably be optimised. Using gene transfer, whether targeted or not, it is (or will soon be) possible to obtain milk containing the best isoforms of normal milk proteins (Mercier and Vilotte, forthcoming). It will also be possible to add to milk for human and animal consumption proteins protecting the newborn and the mammary gland (Houdebine, 1995). Milk will thus become a new nutricentric product. For instance, human lactoferrin can already be produced in cow's milk. Many monoclonal antibodies could protect the digestive tract against viral and bacterial infection. Milk might even contain active vaccinating antigens for oral absorption. The modification of milk composition therefore seems particularly promising.

Figure 6. **Cloned gene applications**

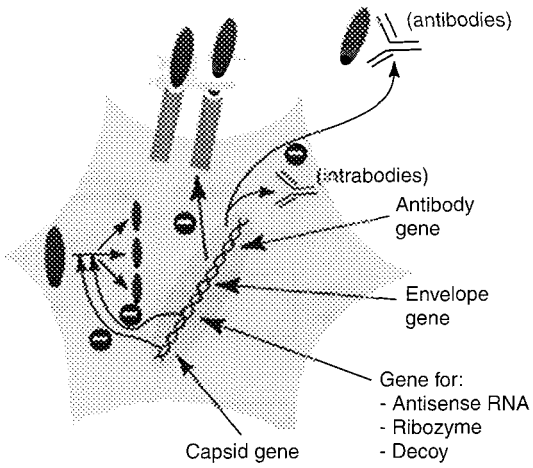


Note: After cloning and, where applicable, mutation, the genes must be decoded using cells to express their messages. Cultured cells can be used for producing proteins for fundamental research or pharmaceutical applications. *In vivo* gene transfer to somatic cells can be used for gene therapy in man or for vaccination purposes. Transgenesis leads to the establishment of stable lines with a new genetic characteristic.
Source: Author.

V. DISEASE CONTROL

Livestock production still suffers major losses through disease. The number of diseased animals can probably be reduced somewhat with less intensive farming practices. In some cases, the disease may be eradicated. Vaccination remains a very effective method and new vaccination tools are now available.

Figure 7. **Disease control through gene transfer**

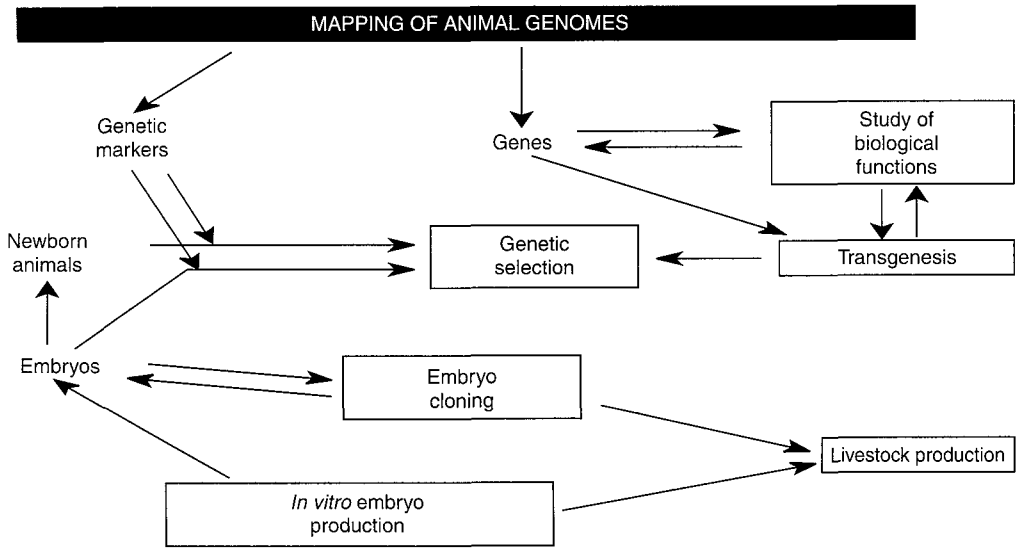


Note: Cellular or viral natural genes (capsid and envelope genes) can oppose the normal multiplication cycle of viruses. Genes directed against the virus' nucleic acid (antisense RNA, ribozymes, decoy) or against its proteins (secreted or intracellular antibodies) can also block the viral cycle. By transferring the corresponding genes to animals it is possible to create genetically disease-resistant lines.
Source: Author.

Viral and bacterial recombinant proteins can now be mass produced. In some cases, the injection of such proteins may lead to the induction of immunoprotector antibodies. For instance, rabbits can be extremely well protected against the streptohaemorrhagic virus (J.F. Vauterot, personal communication). Naturally pathogenic viruses modified through genetic engineering techniques can become both highly safe and effective vaccinating antigens. Viral vectors, and in particular pox virus, which shelter genes coding for pathogen proteins can be used to infect animals in a highly controlled and safe manner, and the latter will then go on to develop effective immunoprotector antibodies.

Recent experiments have unexpectedly shown that naked DNA introduced into somatic cells can be highly effective in inducing the formation of immuno-protector antibodies provided the DNA contains a functional gene. The DNA initially introduced by direct injection into the muscle can also be projected into the tissue by means of the apparatus already routinely used for vaccination with protein or inactivated pathogen vaccines. This new approach offers considerable scope. It seems efficient and relatively straightforward to implement on a large scale. The conditions of use must be standardised to ensure reproducibility and harmlessness. Foreign DNA must not be allowed to appear in organs for human

Figure 8. **Co-ordinated use of genome mapping, embryo cloning and transgenesis to improve livestock production**



Source: Author.

consumption in an uncontrolled manner. The use of RNA, which is readily degradable but more tricky and expensive to handle, would overcome these potential drawbacks.

Transgenesis can also be invaluable in protecting animals against disease. Genes making the host animals resistant to disease can be transferred. These can be natural resistance genes, some of which have been identified but not yet fully characterised and isolated. Protector genes can also code for antisense RNA, ribozymes, monoclonal antibodies whether secreted or not, and also viral proteins (envelopes and capsids) which one way or another block the normal multiplication cycle of pathogens. Limited success has been obtained on animals in this area. This is obviously largely due to the relative difficulty and cost of obtaining transgenic domestic animals. Pigs expressing the MX gene have, for instance, turned out to be well protected against the influenza virus. This approach is therefore relatively cumbersome but it is potentially highly effective since it leads to the creation of animal lines that are genetically disease-protected. The successful use of these methods in plants suggests that they might also work for animals.

VI. USE OF ANIMALS AS MODELS FOR BIOMEDICAL STUDIES

Several animal species are traditionally used as models for research on human diseases and the development of new medicines. Gene transfer has opened promising new avenues in this area. Whether targeted or not, gene transfer may induce in an animal a disorder similar to human disease to a greater or lesser extent. The mouse is by far the most frequently used species for this purpose. However, it does not satisfactorily meet all research requirements. Rabbits which have received human genes to make them hypersensitive or, on the contrary, hyper-resistant to cholesterol are better models for studying atherosclerosis than mice. Rabbits which can be infected by HIV have also been obtained. Transgenic rabbits are also good potential models for studying certain heart diseases, skin cancer, etc.

The transgenic pig seems to be the best potential source of organs for transplant into human beings. The pig organs must be protected against the extremely acute reject response triggered very soon after they are grafted into other species, and especially primates. Pigs expressing genes inhibiting the activation of primate complement by antibodies have been obtained by various researchers. The hearts of such pigs transplanted into monkeys (*Macacus*) survived for two months without showing any significant necrosis. This highly spectacular result suggests that xenografting pig organs into human beings is not impossible.

No matter how important the use of animals might be for biomedical studies or applications, the impact of these new practices is likely to remain negligible for stockbreeders.

VII. SOCIAL IMPACT OF ANIMAL BIOTECHNOLOGY

The growth of animal biotechnology has sparked off a whole range of conflicting responses, from enthusiasm to hostile rejection, and continues to do so. However, the reality of the situation calls for more moderation and equanimity.

The exploitation of animals is becoming increasingly less acceptable to public opinion. Nevertheless, animal research will not be superseded for many years yet. It is impossible to do without animals for many fundamental and applied research projects. Even though it is declining, animal consumption for food purposes will not be phased out in the foreseeable future. The human species is omnivorous through a long process of evolution and there are no grounds to suppose that massive numbers of its members will turn into fully-fledged vegetarians. There is no doubt that it is difficult to define standards governing the conditions in which animals should be used. Such standards can only be established

through consensus, to be called into question as often as necessary while carefully avoiding any kind of anthropomorphism as this obscures the debate without improving the fate of animals. In view of all this, the patenting of transgenic animals should solely be the subject of scientific and technical review to evaluate the inventiveness of the use of such animals. Ethical committees unconnected with the patent filing process could act independently to restrict the exploitation of patented animals on a case-by-case basis.

Many would agree with the philosophers that the dignity of animals is meaningless. However, animals do have rights which must be respected: the right to move, feed and reproduce without any major constraint. One can also readily agree that experimental animals suffer more than animals bred for food consumption. Experimental animals are used in relatively small numbers for cognitive and non-market purposes. The welfare of livestock animals must be more strictly controlled. It should be noted in this connection that, contrary to what some might believe, biotechnology has had very little impact on the status of animals. It can safely be said that the vast majority of transgenic animals do not suffer in any way from hosting a foreign gene. The converse would be surprising. Transgenic animals have only one or a few additional or mutated genes, which is very little compared with their 100 000 genes.

Some people are reluctant to accept the idea of consuming animals hosting genes from other animals or of human origin. There do not seem to be any serious grounds for this reluctance. Any blood transfusion or organ transplant is accompanied by the massive transfer of the donor's 100 000 genes. Furthermore, each day every one of us absorbs billions of copies of at least one million plant, animal and bacterial genes in food, obviously without harmful effects.

The idea of manipulating living organs is fundamentally unacceptable for some, who consider it to be a breach of nature's order. However, this overlooks the fact that living organisms are not stable but, on the contrary, constantly evolving, although this cannot be observed by an individual during his short lifetime. Nature's order is therefore no more than a highly relative concept. Transferring a gene to an animal is simply a clever way of using the laws of nature, and does not consist in changing them. In any case, we are used to manipulations on a much greater scale, even if we are not always fully aware of them. For instance, breeding has generated many different dog races bearing little resemblance to one another although they remain members of the wolf species. No-one is shocked at the sight of a mule, even though it is the outcome of the total and blind transfer of the donkey's 100 000 genes to a horse. No-one would be shocked if a new animal species had been created in this way. The vast majority of biologists consider that conventional breeding, which is a kind of genetic manipulation conducted totally in the dark, is fundamentally more dangerous than transgenesis, which consists in transferring a known gene in a controlled manner.

In this field, the worst is in no way ineluctable. Not all dog races are masterpieces, but these animals cannot be regarded as monsters created by man. To take another example, who would deny that man has successfully used the wild dog rose to fill gardens with roses without spreading thorny bushes through the countryside? What is so feared is therefore the newness of the situation rather than the risks actually entailed. Would there be any sense in suggesting that iron and steel plants should be closed down and iron ore discoverers condemned on the grounds that iron is used to make weapons? Biotechnology undoubtedly entails risks, but most are in no way fundamentally new. Nature has had plenty of opportunity to generate the most virulent pathogens, and any action taken by researchers could not create fundamentally very new situations. Protection against experimentally obtained pathogens therefore does not pose any truly new problem and it is not very difficult to take protective action.

The obtention and use of genetically modified organisms entails real environmental risks. A herd of transgenic cows can easily remain under the control of farmers. On the other hand, transgenic aquatic animals can colonise huge spaces without always being controlled. Such practices may create highly damaging situations. These may be fundamentally no worse than the dangers generated by introducing species from one continent into another. The damage resulting from such practices is well-documented.

Genetic engineering operations are therefore not all equivalent. We must consequently further our knowledge of these fast-expanding topics in order to evaluate the risks which exist, but also to assess the considerable potential benefits. A start was made on drawing up biosafety and bioethic rules before biotechnology became a tangible reality. This is in striking contrast with the growth of the chemical industry or the exploitation of nuclear power. On these grounds, peace of mind and optimism should prevail. There is no reason to deny mankind the benefits of animal technology: we should merely control its use – not an insurmountable challenge.

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ENVIRONMENTAL BIOTECHNOLOGY FOR THE MILLENNIUM

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This article was written by Dr. Mike Griffiths and Dr. Paul Hesselink. Dr. Griffiths manages a major environmental biotechnology research programme on behalf of the UK Department of Trade and Industry. Dr. Hesselink was until recently Head of Environmental Biotechnology for Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (Netherlands Organisation for Applied Scientific Research) in the Netherlands. Together with Professor Ron Atlas, they authored the OECD report, *Biotechnology for a Clean Environment. Prevention, Detection, Remediation.*

I. INTRODUCTION

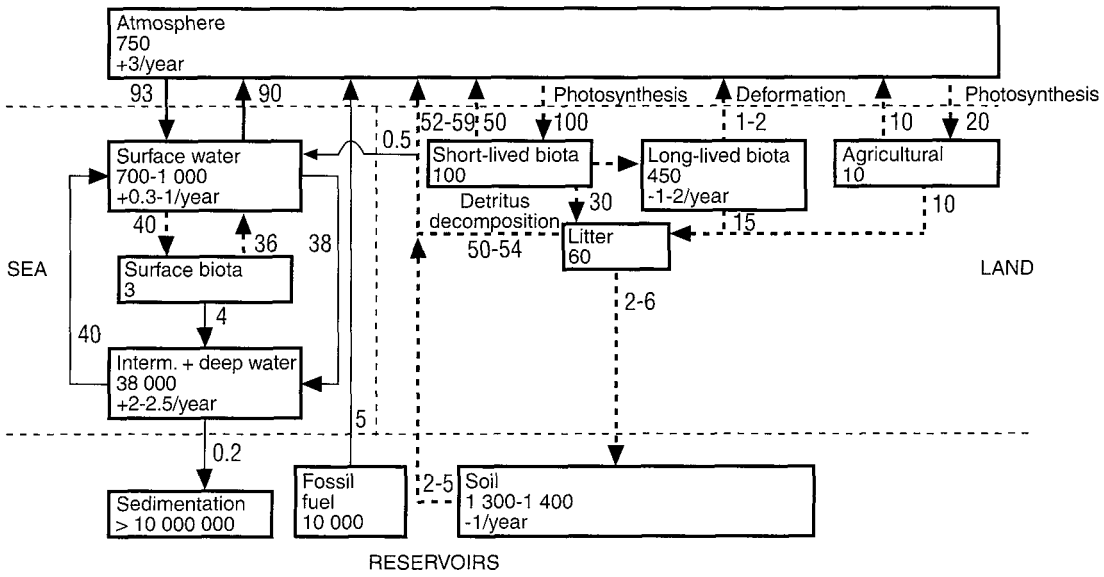
With the imminent arrival of the year 2000, it is appropriate to examine the contribution that biotechnology may be able to make to an improved environment and to future, sustainable development in the next century. Of course, a major use of this technology will still be in cleaning up the pollution and effluents that are with us today and that present day industrial processes will continue to produce. This article will address novel developments which will improve on current processes. In the main, however, we will be concerned with the role of biotechnology in preventing pollution by *i)* substituting conventional processes with cleaner variants; *ii)* replacing fossil fuels and derived raw materials with renewable alternatives; and *iii)* in ameliorating climate change. Additionally, we will try identify where research is needed and speculate on what may be achieved in the coming decades.

Awareness of the damage man can, and is doing, to the environment is spreading rapidly. The use of fossil carbon introduces carbon dioxide, the major greenhouse gas, into the atmosphere; many chemicals in common use both pollute the air, water and soil, and can start a chain of damaging reactions giving rise to long-term unforeseeable effects. In this context, the terms "environmentally friendly technology", "green products and processes", have been receiving increasing attention. These terms refer both to ways of minimising the impact of human activities on the environment and of preventing pollution by increasing energy efficiency or streamlining production processes, and also to new methods for effectively recycling resources and wastes. For example, increased use of renewable resources decreases the need for disruptive mining and drilling operations, and improved plants may require reduced inputs of fertilizers and pesticides. Many of these technologies fall into the category of biotechnology, by which we mean the application of biological organisms, systems and processes to the provision of goods and services.

Bioremediation is the most visible contribution of biotechnology to environmental care and sustainable development. The technology is used to remove, degrade or detoxify pollution in water, air, soil and solid waste by applying the intrinsic cleaning and conversion ability of nature: for example in domestic waste water treatment processes, now more than a century old, in composting and in the use of peat-based biofilters for odour removal.

Biomass consists of lignocellulosic materials including wood and plant materials, the wastes from agriculture, forestry and domestic organic refuse. Its usefulness as a source of energy and raw materials derives from the carbon dioxide (CO_2)-neutral nature of its production and application. Atmospheric CO_2 is the

Figure 1. **Global carbon cycle: Estimated annual fluxes**



Note: Fluxes that can be influenced by biotechnology are presented with dashed lines.
Source: IEA/OECD (1994), *Scoping Study: Energy and Environmental Technologies to Respond to Global Climate Change Concerns*, Paris.

primary source of all the carbon in living organisms. Combustion and degradation of tree- and plant-produced materials thus merely recirculates to the atmosphere the carbon incorporated during the growth of the original biomass, unlike combustion of fossil fuel, which transfers to the atmosphere CO_2 trapped in plant and animal material over geological time spans (Figure 1).

Biotechnology can be used in a number of other ways to prevent environmental damage: for example, by using a biotechnological process to convert a waste stream into value-added products. Process innovation, the development of new, biological, processes and the modification of existing ones by the introduction of biological steps, is one option, and the use of new biofuels and biomaterials, which have little or no environmental impact, *e.g.* biodegradable polymers, is another.

II. REMEDIATION AND WASTE TREATMENT

Sure, those bugs can eat it all, but will they?", Witholt and Hesselink, University of Groningen.

Since the beginning of modern biotechnology, environmental applications have been regarded as one of the three potential success areas, along with agro-food and health care. However, developments in the environmental sector have taken place at a slower rate, in part because of weaker market incentives and a lack of strong R&D support. Two other factors have also contributed to this slower development. Firstly, there has been a lack of coherent environmental legislation and enforcement; and secondly, treating waste has long been less glamorous, being seen as a cost, rather than a source, of profit. However, growing public concern about the impacts of waste discharge and pollution and the clear need for a more sustainable way of life, have led to more attention from both the public and politicians to environmental care. Science-push is being replaced by societal need and a stronger market-pull for innovative solutions.

Recent progress and developments

Since the early 1980s, bioremediation has seen rapid progress in fundamental science and technology. The first reason for this is the realisation that many more contaminants can be efficiently degraded by micro-organisms than had been thought. Until the late 1970s, chlorinated hydrocarbon solvents were considered as persistent and non-biodegradable; a situation which has changed with the elucidation of new microbial pathways for xenobiotic biodegradation and a better understanding of how these could be applied and optimised. Table 1 lists the many groups of organic compounds amenable to bioremediation.

Table 1. **Chemicals potentially suitable for bioremediation**

Class	Example	Aerobic process	Aenarobic process
Monochorinated aromatic compounds	Chlorobenzene	♦	♦
Benzene, toluene, xylene		♦	♦
Non-halogenated phenolics and cresols	2-methyl-phenol	♦	♦
Polynuclear aromatic hydrocarbons	Creosote	♦	
Alkanes and Alkenes	Fuel oil	♦	
Polychlorinated biphenyls	Trichlorobiphenyl	♦	
Chlorophenols	Pentacholorophenol	♦	♦
Nitrogen heterocyclics	Pyridine	♦	
Chlorinated solvents			
Alkanes	Chloroform	♦	♦
Alkenes	Trichloroethylene	♦	♦

Source: Environmental Protection Agency, United States.

The second development has been the improvement in bioreactors and processes. Unlike most other applications of biotechnology, bioremediation takes place in so-called open systems. This means that there are no sterile reactors, inoculated with single microbial strains and under stringent control of parameters such as temperature, pH, oxygen supply and nutrient addition. By contrast, in an open system there is strong competition between many micro-organisms for nutrients and many strains of organism are present including ones that predate on others. Oxygen supply may be limiting, pH varies greatly, as does temperature, with time (day-night, seasons). Even the pollutants are not abundantly available in free form as they are often adsorbed onto, or absorbed in, solid particles and are difficult for the micro-organisms to reach. This is commonly referred to as the problem of bioavailability. Only recently have biotechnologists managed to create more stable operations in their reactors and processes, thus paving the way for more reliable and predictable operations. However, these processes are often still based on a "black box" approach rather than on sound insight into the underlying principles and ecological interactions involved.

Bioremediation is now offering solutions to many problems. These solutions are real: pollutants are totally degraded into products that form part of natural material cycles. There is no accumulation of contaminants nor are they transferred from one natural compartment to another, such as the movement of hydro-

Table 2. **Costs of biological and non-biological treatment of environmental pollution (soil, air, water) in the Netherlands**

Soil remediation	Gld/tonne	Air treatment	Gld/ 1 000 m^3	Water treatment	Gld/m^3
In situ:					
Bioremediation (BT)	70-150	Biofiltration (BT)	0.50-5.00	Biological water treatment (BT)	0.10-1
Extraction	125-150	Bioscrubbing (BT)	3.00-6.00	Sedimentation	0.05-30
Electro-reclamation	150-300	Chemical scrubbing	1.00-> 20.00	Flotation	0.10-2
Stream-stripping	250-300	Adsorption (activated carbon)	1.00-10.00	Adsorption	1.00-10
		Incineration	2.50-25.00	Chemical oxidation	0.50-> 5
		Catalytic treatment	2.50-20.00	Ultra-filtration	< 1.00-> 20
On/off site:					
Land-farming (BT)	50-140				
Extraction	120-240				
Thermal treatment	100-300				

Note: BT = biotechnology.
Source: OECD (1994), *Biotechnology for a Clean Environment: Prevention, Detection, Remediation.*

carbons from water into air as happens in "stripping" processes. Furthermore, in contrast to most physical or chemical environmental technologies (such as incineration, scrubbing, catalytic conversion or precipitation), bioremediation rarely requires input of energy or chemicals and generates no secondary wastes (such as ashes and dioxins, polluted scrubbing liquids and chemical wastes). As a consequence, bioremediation is often an attractive and cheap solution (Table 2).

As a result of this scientific and technological progress, bioremediation has begun to obtain a substantial share of the total environmental market over the last decade. At present, some 20-30 per cent of the total environment market consists of products or services with a major biotechnology component. This equates to an annual turnover of US$50-75 billion. The growth rate of this market is about 10 per cent annually, whereas the total environmental technology market is growing at 4-5 per cent per year.

Biotreatment of water, air and off-gases

Recent improvements in water treatment processes have made it possible to treat carbon-based wastes, nitrogen, phosphorus and sulphur compounds and xenobiotic organic compounds such as solvents, pesticides and aromatics, in a very efficient and cost effective way. This includes both domestic and a wide variety of industrial waste waters.

A major drawback of aerobic water treatment has been the production of secondary wastes; the most important being sewage sludge and nitrous oxide (N_2O). Sewage sludge consists of the remains of micro-organisms which carry out the treatment. Its composition could make it an excellent agricultural fertilizer and land improver but it is often contaminated with heavy metals and other pollutants. Also, the amounts of sewage sludge generated during conventional aerobic treatment are rather high. Future improvements will lead to both the minimisation of sludge production and its decontamination.

For N_20 the problem is rather more complex since it is a very potent greenhouse gas with much stronger effects on global warming than an equivalent amount of carbon dioxide. It is generated during the removal of nitrogen compounds from waste water, and inhibiting production will require the use of alternative micro-organisms and biodegradative routes, and a better understanding of the interactions between the various microbial strains involved in this part of the biological nitrogen cycle.

Biological air and off-gas treatment has long been synonymous with the use of compost and peat-based biofilters for odour removal. Over the last ten years, however, there has been considerable progress and currently many industrial off-gases can be biologically treated. This has been achieved by the optimisation of

the microbiological systems in classical compost biofilters and by the use of other carrier materials such as ceramics, polyurethane, perlite and plastic rings. More specialised micro-organisms with good performance under extreme conditions have been identified and applied, resulting in, for example, a perlite-based biofilter, using fungi active at low pH (1.5-3.0) and low water activity, for the efficient and stable removal of styrene from industrial off-gas. Another successful innovation has been the development of bioscrubbers and biotrickling filters. Such reactors rely on an intimate contact between the gas phase with pollutants, and the aqueous phase with immobilised or non-immobilised micro-organisms. This has yielded compact reactors with better options for control than biofilters, but the cost of installations and operating them is significantly higher. Other major improvements will be the result of combining or integrating biological with physico-chemical reaction steps. For instance, a photocatalytic pre-treatment will crack persistent molecules into molecules which can be rapidly and efficiently degraded by micro-organisms in a biofilter. An example of such a combined technique is the biomembrane reactor. Here, the contaminants are separated from the bulk main gas stream by membrane filtration. The micro-organisms, on the other side of the membrane, or possibly in another reactor, can mineralise the concentrated contaminant in an efficient way. Tests have already indicated that such an arrangement is reliable enough for extended application in space shuttles for the removal of pollutants from the shuttle crew's air supply.

Biotreatment of soil, sediments and solid waste

There are clear differences between the bioremediation of fluids (water and off-gases) and the clean-up of solids. Firstly, contaminants are relatively homogeneously distributed in fluids while pollution in soil or solid waste is very heterogeneous, ranging from negligible to concentrations unacceptably high for microbial action. In tar clumps, for example, poly-aromatic hydrocarbons (PAHs) can constitute nearly 100 per cent of the solid mass. Secondly, the pollutants may be only poorly available to the micro-organisms and bioremediation will therefore proceed at a very low rate, be incomplete and result in residual pollution concentrations. Thirdly, there is a mixing problem when essential nutrients or oxygen have to be transported into the solid matrix for biodegradation to occur. Some of the mixing and heterogeneity problems can be diminished by making aqueous slurries of the solids, but this is relatively costly and does not solve the problem of bioavailability.

During recent years, much progress has been realised in bioremediation of soil *in situ*. Clean-up of soil contaminated with a range of organic pollutants can be demonstrated following percolation with water containing nutrients and oxygen

and treatment of the contaminated (ground-)water in bioreactors. Stimulation of the endogenous microbial population, at the same time pumping in air or steam, together with addition of essential nutrients and oxygen, has achieved good clean-up. The polluted air coming out of the soil is treated using biofilters. Another promising option is a combination of aerobic and anaerobic process steps. This opens the way for, for example, the cracking of a persistent contaminant in a series of anaerobic reactions followed by total rapid aerobic mineralisation. Polychlorinated biphenyls (PCBs), dioxins, complex PAHs and other chlorinated compounds may be efficiently removed by such a strategy. Improvement of solubility or bioavailability by pre-treatment with exo-enzymes could be another way to improve biotreatment of polluted solid matrices. Most of these options are currently being investigated and tried in field studies. Some will yield insights or solutions for the bottlenecks mentioned; others will serve as platforms for innovation.

Similar solutions are being developed and tested for the biotreatment of solid wastes. In addition to improved aerobic composting, anaerobic composting has also been developed, sometimes in combination with so-called slurry digestion. Organic waste is anaerobically treated after fractionation in more or less conventional anaerobic waste water installations. A "dry" anaerobic process has also been developed which uses the water present in the waste. Variations of these processes can be applied to other high organic wastes such as manure, agricultural and domestic wastes.

Heavy metals constitute a serious and widespread problem. As these cannot be degraded, they can only be converted, displaced or concentrated. Biotechnology offers potential solutions by bioleaching and bioaccumulation. During bioleaching, metals are removed from their precipitated and immobilised state by acid-producing micro-organisms. Successful applications of this principle can be found in biomining for the production of copper, for example. Accumulation of metals can be achieved in many ways. Some plants have shown great ability in removing heavy metals from soil, and several micro-organisms have shown strong affinity for selective accumulation of specific metals. These potentials, as with many others in bioremediation, will have developed into practical techniques within one or two decades.

Priority targets for R&D in bioremediation include:

- identification, development and characterisation of micro-organisms which readily degrade a broader range of pollutants;
- identification, development and characterisation of micro-organisms able to function under more extreme conditions with respect to temperature, salt tolerance, concentrations of pollutants, fluctuations in physiological conditions and the presence of toxic compounds;

- mixed pollutant biodegradation or biodegradation of target pollutants in the presence of other, more readily biodegradable substrates;
- optimisation of the rate of bioremediation, especially in treatment of soil, sediments or solid waste;
- finding solutions for the low bioavailability of pollutants in soil, sediments and solid waste;
- lowering the residual contaminant concentrations after biotreatment of heavily polluted soil, sediments and solid waste;
- integration of different biotechniques or biotechniques with physico-chemical techniques in one process, or the development of biomodules to be coupled to other modules for pre-treatment or polishing steps to provide cost-efficient overall solutions;
- increasing the reliability and predictability of bioremediation with respect to operational stability and treatment results under fluctuating conditions in an open environment.

III. PROCESSING AND MANUFACTURING

"The traditional view is that if you can't order it from Sigma, it's just not real", Gregg Whited, Genencor.

Conventional chemical manufacturing processes require substantial energy inputs and generate large amounts of waste and by-products through the use of high temperatures, extremes of pH, a wide variety of highly reactive chemicals and organic solvents in order to achieve high conversion rates. Cleaner technology addresses the replacement of these conventional processes by biological processes. Biotechnological processes generally occur at moderate temperatures (10-60°C), moderate pH levels and in aqueous systems. Biological systems are highly selective, the use of additional chemicals is minimal and there is little waste in the form of by-products, especially inactive forms of the primary product.

An example of a replacement process is the use of a biological reaction in the manufacture of a major industrial chemical, acrylamide. Acrylamide is produced industrially as a monomer for synthetic fibres, flocculating agents, etc. The conventional synthetic process involves hydration of the nitrile with sulphuric acid and/or the use of inorganic catalysts. The inclusion of the enzyme (a biological catalyst) nitrile hydratase in an alternative route avoids several costly steps, is the first case of such a replacement and is possibly the first use of biotechnology in the petrochemical industry.

Biocatalysts

Biocatalytic reactions offer many advantages, particularly for the synthesis of fine chemicals. Conventional processes often give rise to so-called racemic mixtures of chiral isomers (left- and right-handed versions) of the product, only one of which may be useful or chemically active. The inherent selectivity associated with many enzyme-catalysed reactions means that they often lead to the production of a single isomer, thereby minimising complicated separations in downstream processing.

Hydrolysis reactions were among the first to be substituted by biological alternatives but reduction reactions are also being replaced. A widely used enzyme in this respect is the yeast-derived alcohol dehydrogenase although, such is the speed of development, more robust, more active versions of this enzyme will shortly be available from other sources. Another functional group transformation catalysed by yeast is the reduction of carbon-carbon double bonds. In the 1980s, Hofmann LaRoche in Basel, developed a commercial process using baker's yeast to reduce the double bond of a cyclic α, β-unsaturated ketone in the key step. This reduction gave a pure chiral ketone while achieving considerable cost savings over the existing chemical conversion.

Organisms with alien biochemistries are now being collected from the most harsh environments on earth – in the depths of oil wells, in arctic ice, in desiccating salt marshes, and above thermal vents on the deep ocean floor. The enzymes, so-called extremozymes, from these organisms are of interest because, while we have yet to devise a conventional catalyst as selective as an enzyme, the downside is that enzymes from ordinary micro-organisms are often too delicate to be of use in most industrial processes. In other words, the operational stability of enzymes under practical conditions requires enhancement. Process engineering is looking to these extremozymes to combine exquisite precision with the toughness needed to survive in industrial processes.

Recent research has shown that enzymes do not need to be kept in aqueous reaction media to catalyse technologically useful reactions. This has led to a new form of enzyme biotechnology in which lipases, proteases and carbohydrases (enzymes which normally hydrolyse lipids, proteins and carbohydrates) are controlled by water depletion, and persuaded to form the chemical bonds they would normally break. This is the basis of emerging methods for modifying the functionality and nutritional properties of, for example, food fats, and the synthesis of food emulsifiers and flavours. Water itself is not an ideal medium for synthetic purposes, since it often participates in the side reactions and may complicate product recovery. Hydrolases generally display a substantial relaxation of specificity in anhydrous conditions and, as a result, accept a variety of very unusual substrates. For example, subtilisin, an enzyme whose natural function is protein

hydrolysis, readily catalyses the addition of acidic sub-units to sugars in organic solvents to yield surface-active food emulsifiers.

The need to eliminate solvents altogether from some food compatible reactions has led to a further remarkable discovery in this field: that enzymes can operate even under solvent-free conditions, with only substrates and products as their bioreaction environment. While many enzymes generally do not survive in organic solvents, and a high proportion of those that do survive become tens of thousands of times less active, a few enzyme-catalysed reactions actually seem to proceed better in non-aqueous media than in water. The advantages of using non-aqueous media include increased solubility of non-polar substrates and elimination of microbial contamination. Another incentive to use enzymes that can withstand organic solvents is that these solvents make the enzymes more selective about the targets they bind to. One reason for this change in selectivity is that solvents change the flexibility of the protein chain which, in turn, can control the way the enzymes select their targets.

There is no natural environment that mimics an organic solvent and thus is a source for appropriate organisms. However salt, like solvents, dehydrates enzymes, sticking to them and shielding them from water and therefore, to improve the level of activity in solvents, research groups are looking at extremozymes from halophilic (salt-tolerant) bacteria. These organisms can survive in water which has 30 times the salinity of the sea and their enzymes actually require very salty water to function. An halophilic enzyme freeze-dried from salt water resulted in a powdered salt-enzyme combination which functioned almost as well in organic solvents as it did in its natural environment.

Novel enzymes

Research into protein engineering is beginning specifically to lead to the alteration of the amino acid sequences of enzyme proteins in such a way that their folded 3-D structure acquires more stability to industrial conditions, and/or confers an altered substrate preference closer to commercial needs. Ultimately, manufacturers will thus be able to take advantage of the high selectivity of enzyme-catalysed conversions under non-physiological, commercial conditions.

Developments such as these have been made possible by some remarkable improvements in the understanding of enzymes at the molecular level. For example, the enzyme xylose isomerase is inactivated by a chemical reaction between glucose (its substrate) and the secondary amino groups of the amino acid lysine which are critical to its three-dimensional structure. Researchers at Gist Brocades have found a way a modifying the gene for the enzyme so that the lysine units are replaced by less reactive groups (arginine units), which will still hold the enzyme

structure together. The consequence is that the glucose no longer reduces the catalytic activity of the enzyme.

Natural extremozymes resistant to heat and pressure are being found in organisms from the deep-sea. The rewards for their discovery can be high, as the example of the so-called taq polymerase shows. This enzyme, from the thermophilic bacterium *Thermus aquaticus,* is the basis of the polymerase chain reaction (PCR) which catalyses the rapid multiplication of pieces of genetic material. The enzyme has revolutionised entire areas of biochemistry, from the treatment of disease to genetic fingerprinting, while making hundreds of millions of dollars in sales. Furthermore, taq polymerase is not considered a true extremozyme; although it is hardy enough to survive the repeated heating and cooling steps of the standard PCR, it breaks down at the "cool" temperature of 80°C. Researchers are now experimenting with an improved version of PCR using hardier enzymes from deep-sea vent bacteria, where pressure allows the water temperature to reach 120°C.

The unusual properties of extremozymes may lead to entirely novel chemical processes. For example, while attempting to express the gene for an enzyme from a heat-vent organism in *Escherichia coli,* it was found that the gene coded for a much larger protein which was inactive at low temperatures but which split apart, spontaneously producing new enzymes and three other fragments that spliced themselves when heated to form a further enzyme.

Future targets for R&D in this area include:

- identification and selection of novel, extremophile organisms; exploration of their metabolic pathways and enzyme structures; identification and transfer of genes for the more active enzymes;
- a better understanding of enzyme structure/function relationships, especially in relation to non-aqueous systems;
- immobilisation of biological systems (enzymes and organisms) on a range of substrates.

IV. MATERIALS

Many industrial chemicals and products of chemical manufacture in common use are carbon-based and derive from fossil carbon sources, primarily crude oil and natural gas, which are finite resources. When these manufactured materials reach the end of their useful life, if they cannot be recycled, they are often incinerated or left to degrade, giving rise to CO_2 and other polluting compounds.

Renewable materials

The parallel universe of chemicals and materials production, based on biomass raw materials and entirely separate from that based on fossil carbon, is hardly being explored, in part because of the complex chemistry involved, but also because of the relative costs of the raw materials. Nonetheless, a wide range of non-food, non-energy products are currently derived from biomass. To give a few examples: cotton production for fibres exceeds all synthetic fibres; natural rubber competes in quantity with synthetics; starch is used as an additive in paper and textiles and as an adhesive; vegetable oils are made into soaps and detergents. In future such bioprocessing will include the modification of structure and function to improve product design and produce such novel biomaterials as superior silks and spider webs.

Perhaps the most attractive feature of biomass, which is a lignocellulosic material, is that it is a product of photosynthesis and, as such, is a CO_2-neutral renewable resource. The structural components of biomass vary from source to source, but lignin, cellulose and hemicellulose are always present. The US pulping industry produces 20 million tonnes of lignin as a by-product of paper-making. Virtually all of this lignin – the second most abundant biological polymer on Earth – gets burned as waste. Wood scientists, who think of wood as three-dimensional biopolymer composites, want to see this resource become a supply of high-technology materials, including plastics. Lignin's complicated and only partially understood chemical structure has so far discouraged researchers from developing a routine chemical basis for its exploitation. However, the lignin that emerges from the pulping process appears to follow some structural rules. For example, lignin components of specific molecular sizes link and dissociate in a particular order and, using this knowledge, it has been possible to develop methods for casting films made of the lignin biopolymer.

Further attention to the relationship between wood structure while growing and the physical properties required of constructional and other materials has led to the possibility of genetically engineering trees to meet a much wider requirement. Novel wood-based materials can replace components made from fossil fuels and at the same time effectively sequester CO_2. Reduction of lignin in tree species intended for pulpwood has already been achieved.

Use of a larger number of plant species is also occurring. For example natural rubber is presently derived from only one tree species – *Hevea*. There are, however, more than 300 other genera-producing isoprenes, many yet to be exploited.

Genetic technology will dramatically change the materials available from biomass. Recent advances in recombinant genetic biotechnology of soya beans have led to ways of altering the lipid composition in order to increase the variety of

biohydrocarbons available. In addition, the yield, structure and degree of saturation of the oils from soya and other vegetable sources will be modified. The application of genetic engineering to cotton is in its infancy despite the high commercial importance of the crop. Cotton requires some 10 per cent of the world production of agrochemicals, in the main pesticides, and the introduction of insect resistance could save huge quantities of polluting chemicals.

An important renewable resource is the soya bean, whose versatility as a raw material ranges from food products and diesel fuels to polymers, fabric softeners and solvents. Several hundred industrial products made from soya beans were developed in the 1930s and 1940s, including adhesives, rubber substitutes, printing inks and plastics. Amides, esters and acetates of biohydrocarbons are currently being used as plasticisers, blocking/slip agents and mould-release agents for synthetic polymers. Biohydrocarbons linked to amines, quaternary ammonium ligands, alcohols, phosphates and sulphur ligands are used as fabric softeners, surfactants, emulsifiers, corrosion inhibitors, anti-static agents, hair conditioners, ink carriers, biodegradable solvents, cosmetic bases and perfumes. Complexes with aluminium, magnesium or other metal compounds have produced greases and marine lubricating oils.

Soy protein and sugars may be used in the production of polyurethane foams for packing, insulation and padding. Using these materials to replace expensive polyalcohols improves the biodegradability of these materials as well as reducing the need for environmentally undesirable fluorocarbon foaming agents. Co-extrusion of soy protein with polyvinyl chloride produces a silk-like fibre, with greatly increased wet/dry tensile strength, while retaining wearing comfort, as a result of the hydrophilic nature of the protein fibres.

In addition to producing new chemicals from biomass, there exists the possibility of using photosynthesis directly: the aim being to create an artificial system mimicking photosynthesis to convert CO_2 to hydrocarbons and other organics. Microbes, both land- and sea-based, remain a huge untapped resource of metabolic diversity – biosynthetic pathways, new drugs, polymers, etc. In the long term, atmospheric CO_2 may be a significant raw material for these products.

Biodegradable plastics

The route to sustainable development lies through optimising the recycling of all materials. Plastics in particular are so widely used that considerable interest and effort has gone both into direct recycling and the development of varieties that can be "recycled" by biodegradation.

The structure of a molecule is key to its biodegradability. In general, polymers with mixed backbone linkages (carbon-oxygen or carbon-nitrogen) show greater

susceptibility to hydrolysis than carbon-carbon backbone polymers. Polymers with aromatic components or branched regions tend to be more resistant to attack than straight-chain aliphatic components. The primary biological mechanism for degradation of high-molecular-weight polymers such as plastics is hydrolysis by extracellular enzymes produced by micro-organisms. To be biodegradable, the polymer chain must be flexible and have a stereo configuration that allows it to fit into the active site of a degradative enzyme.

A general class of biodegradable plastics are the microbially-produced polyesters which have ester bonds that are susceptible to enzymatic attack. These compounds include poly-(betahydroxyalkanoates), or PHAs, an example of which is polyhydroxybutyrate, and polylactate, which has a number of medical uses. PHAs are synthesised by bacteria as a reserve material, and nitrogen and phosphorus limitation can be used to enhance intracellular PHA accumulation. Under the appropriate conditions, the bacterium *Alcaligenes eutrophus,* for example, can accumulate an astounding 96 per cent of its dry weight as PHA.

A completely biodegradable co-polymer, poly (3-hydroxybutyrate-3 hydroxyvalerate) or PHBV, is produced by some types of bacteria. In the early 1980s, Zeneca (formerly part of ICI) developed a process for growing large batches of these bacteria, and harvesting the PHBV. PHBV is renewable, biodegrades rapidly and completely (to carbon dioxide and water) in soil or in a landfill, yet remains stable in storage, is biocompatible for medical devices and personal hygiene products, and can be processed into films and containers using existing technology. The material, known as Biopol, is so far the only commercially produced bioplastic, and even in this case costs and technology need improvement. For example, since the co-polymer is an aliphatic polyester, it is susceptible to hydrolysis in an aqueous medium. Also, since it is so highly crystalline (70 per cent or more), it melts at a fairly well-defined temperature, meaning processing equipment and moulds have to be carefully matched to the resin. Zeneca has recently sold its Biopol business to Monsanto.

In the late 1980s, the applied microbiology laboratory at MIT became the first research facility to bioengineer PHA biopolymers using recombinant DNA. Other researchers have successfully transferred the genes for the three enzymes involved in the synthesis of polyhydroxybutyrate (PHB) into *E. coli,* where they were expressed. Workers at Michigan State University have grown PHB in *Arabidopsis thaliana,* a plant of the rapeseed family. The plastic-producing genes from *Alcaligenes eutrophus* have been inserted into the plant, which then makes PHB granules throughout its leaves, stems and roots. These granules can be recovered for use as a raw material for bioplastics production. Current research is attempting the transfer of the same genes to rape.

There is speculation that inserting the genes for PHBV production into plants may one day simplify biological plastic production by allowing harvesting of crops

from which plastic could be extracted. It could be five years before there is a viable product, and ten years after that before bioplastic crops are in the fields; but there is no reason in theory why crops cannot be programmed to produce a more complex, commercially usable plastic and thereby out-compete micro-organisms as the primary source.

R&D on biomaterials will need to address:

- improved biological production from a wider range of renewable raw materials;
- identification of novel metabolic pathways in land-based and marine organisms;
- understanding of the structure and chemistry of lignin and transfer of genes for lignin-degrading enzymes to common bacteria;
- genetic manipulation of plants to improve productivity of specific materials;
- genetics of PHA production and transferability to plants and faster growing organisms.

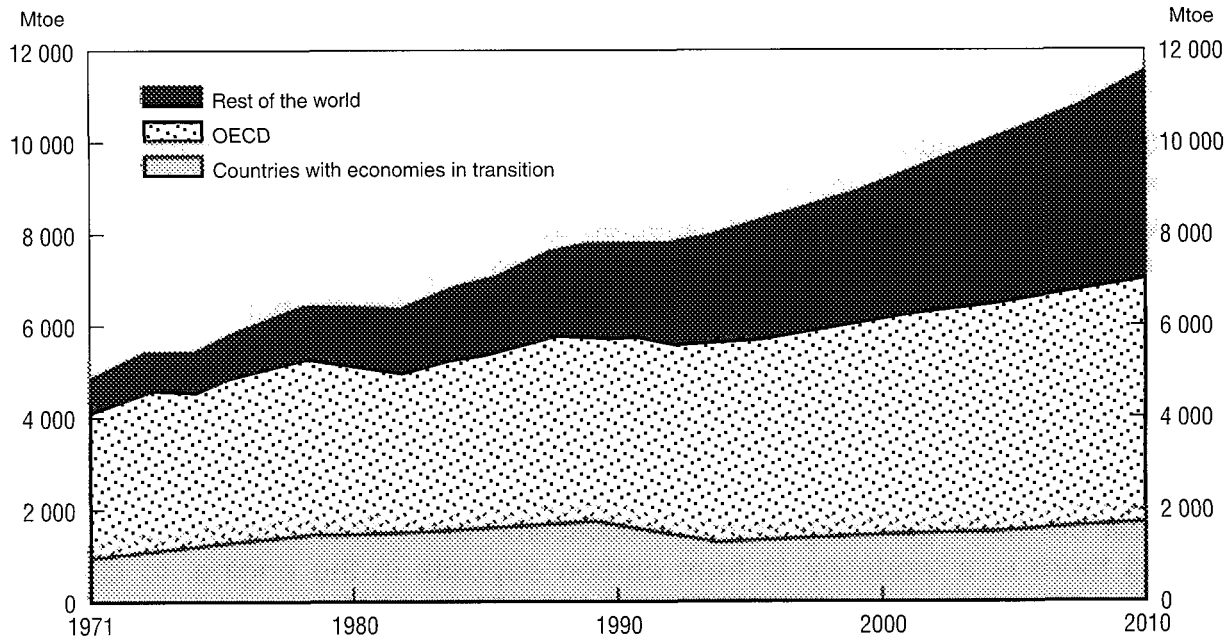
V. ENERGY

"Human beings are now carrying out a large-scale geophysical experiment of a kind that could not have happened in the past nor be reproduced in the future", Roger Revelle and Hans Suess.

The demand for energy rises inexorably, much of it supplied by carbon-based fossil fuels (Figure 2). Biomass, solar power, wind and geothermal energy together account for some 4 million barrels per day oil equivalent (bdoe) or less than 2 per cent of global commercial energy supply. If non-commercial energy is included, biomass is estimated to contribute some 25 million bdoe, equivalent to some 14 per cent of the world's total energy. Much of this non-commercial energy use is in the developing world which depends on biomass energy for more than a third of its total energy requirements.

Biomass, either purpose-grown or waste, can be the raw material for a range of fuels ranging from electricity via several carbon-based liquid fuels to hydrogen. Their merits and de-merits may be compared on economic, environmental and/or fossil fuel conserving (CO_2 abatement) grounds. The factor which links them all is that they are perceived as generally neutral with respect to atmospheric CO_2. As the crop grows, it absorbs CO_2 which is then released when the biofuel is burned, but no more is emitted into the atmosphere than was originally absorbed. This is not entirely true if quantities of CO_2 are produced by farm equipment harvesting the biomass and manufacturing processes converting it.

Figure 2. **World total primary energy demand**



Source: IEA/OECD (1994), *Scoping Study: Energy and Environmental Technologies to Respond to Global Climate Change Concerns*, Paris.

The reluctance to use biofuels commercially is based both on the cost of conversion of power trains for the new fuel and the actual production cost of the fuel. New technologies for production and consumption are slowly bringing these costs to the point where biofuels will compete on the open market, without subsidy, with their fossil fuel equivalents.

Several studies have shown that it is more cost-effective to grow energy crops for use as solid fuels for heat or power rather than for liquid transportation fuels. On the other hand, a transportation fuel and the freedom it grants may have a social value which is not completely reflected in the price. Fuels are valued not purely economically but also socio-economically – for their user-friendly qualities, for instance. Thus, liquid fuels in cars may win out over electricity, but the latter is preferable in most domestic situations.

Transportation fuels

Certain plants contain throughout their tissues latexes of terpene-based hydrocarbons. These plants, which grow in semi-desert conditions would not compete with food crops for land. It has been suggested that these hydrocarbons

be extracted and used as feedstock in conventional refineries. Yields are currently of the order of 7-8 boe (barrels of oil equivalent)/ha/a. In the long term the conversion of total biomass to hydrocarbons by hydrothermal processes is likely to give higher yields of more suitable molecules, more economically.

Some green algae also produce unusually high levels of liquid hydrocarbons under certain restricted growth regimes. These algae occur throughout the world in a wide variety of climates. One selected species yielded approximately 30 per cent of lipids on a dry matter basis, of which up to 70 per cent were hydrocarbons. Like plant latexes, these materials cannot be used directly but would have to undergo a refining process.

A number of plant-derived oils have been considered for possible use as fuels in diesel engines. These include crops such as sunflower, soya, groundnut, cottonseed, rapeseed, palm oil and castor oil. Vegetable oils have been used in the past but have generally been unsuccessful: even when the oils are highly refined there is a tendency to form carbon deposits in the engine, to clog up injection systems, and to produce highly particulate emissions. Diesel engines operating on the fuels have reduced efficiency and higher maintenance requirements.

Vegetable oil esters such as rape oil methyl ester (RME) can be used as such, or in blends with diesel fuel. They have lower viscosities and higher octane numbers than pure vegetable oils and thus have greater potential both as diesel fuel substitutes or blends. Research testing has shown some advantages: exhaust emissions generally contain reduced hydrocarbons and produce less black smoke.

From an economic standpoint, biodiesel, produced from oil crops which have high levels of agricultural inputs, competes very unfavourably with conventional diesel and indeed may not have a favourable energy balance when its total production chain is taken into account. Production from oil palms in tropical countries may, on the other hand, soon become competitive.

Ethanol and methanol

Ethanol produced from sugar and starch crops can be used as a gasoline extender or substitute, or a feedstock to make the octane enhancer ETBE (ethyl tertiary-butyl ether) which may be more highly valued as a gasoline additive. Ethanol is produced from simple sugars by fermentation with yeasts such as *Saccharomyces cerevisiae,* used in bread- and beer-making, and *S. ellipsoides,* used in wine-making. Starch has first to be converted enzymatically to glucose. Methanol produced from woody biomass can also be used as a gasoline extender

or substitute or, in this case, a feedstock to make MTBE (methyl t-butyl ether) which would otherwise be produced using methanol from natural gas.

Alcohols have high octane numbers which makes them suitable for use in spark ignition engines. Engines designed for neat ethanol use hydrated rather than anhydrous ethanol. They can have higher compression ratios (lean burn) and modifications have to be made which make them no longer suitable for ordinary gasoline. Early corrosion difficulties with, for example, carburettors have been solved but there can still be a cold-start problem.

Considerable effort has gone into improving the economics (and technology) of conventional ethanol production with a view to minimising heat consumption and waste production. The major energy requirement is in the conventional distillation process. Distillation requires substantial low-grade heat energy although this can be supplied as waste heat from, for example, a conventional power station or by burning waste agricultural material. The dominant costs, on the other hand, for ethanol produced from starch or sugar crops are for feedstock and capital costs.

Much research has been devoted to increasing productivity via yeast or bacterial cell immobilisation, solvent stripping of the ethanol, ceramic membrane separation of the ethanol, combined fermentation, distillation under vacuum and continuous rather than batch production. Combination of continuous fermentation under vacuum and cell recycle can result in a twelve-fold increase in ethanol productivity over conventional processes. Yeasts are normally used to ferment sugars, although bacteria are being considered with the aim of operating at up to 10 per cent ethanol concentration. Some bacterial species have been characterised which may tolerate concentrations of ethanol as high as 20 per cent.

Short-term process improvements, giving a 10-15 per cent cost reduction in five years, involve:

- replacing yeast with bacteria such as *Zymomonas mobilis* in order to achieve faster fermentation rates, better ethanol and temperature tolerance;
- membrane separation of solubles, which can result in up to 40 per cent water removal prior to distillation;
- immobilisation of organisms, which leads to better control.

Other possible improvements on yeast fermentation, which together could achieve a further 20 per cent cost reduction over ten years, include:

- extraction of ethanol with supercritical CO_2, further use of membranes, variations on vacuum fermentation;
- the use of pelletised yeast in fluidised beds: ethanol/water is stripped out by the inert gas used for fluidisation;

– use of molecular sieves to replace azeotropic rectification.

Although production from starch and sugar crops has limited further potential, mainly because of raw material availability, processes based on other substances have yet to be successful. Lignocellulose, from energy forestry and from agricultural and forest industry residues, has long been considered as a source of biomass liquid production. In contrast to starch crops, the processing energy requirements for lignocellulose can be met using substrate-derived residues with no supplemental biomass or fossil fuels. Compared with corn, lignocellulose substrates are 20 times more plentiful in the United States, and do not compete with food production. Over a 20 year period, total US ethanol production could rise to 470 million tonnes/year from waste (198 million tonnes), forestry (184 million tonnes) and agriculture (corn and grasses, 88 million tonnes), equal to present gasoline consumption in energy terms.

The major problem with woody materials is making the substrate available to the enzymes and organisms which will further ferment it. Some pre-treatment of the lignocellulose is required to increase its porosity and make it available for enzyme or acid attack. Each has disadvantages: enzymes use some substrate for growth, while acid yields degradation products. Traditionally, cellulase from the fungus *Trichoderma reesei* has been used. Cellulase is present in numerous meso- and thermophilic fungi and bacteria. That from *Clostridium thermocellum* has been the most powerful found so far. A new area in environmental biotechnology is the possible use of free-radical-generating enzymes to degrade the complex wood organics. These systems are the basic lignin-degrading mechanisms of the white-rot fungi.

There is the potential for a two-fold decrease in costs using technology based on enzyme or acid hydrolysis and yeast fermentation. Research aimed at wood-degrading organisms with ethanol tolerance comparable to that of yeasts has not yet succeeded, although some mutants more tolerant to ethanol have already been selected. There is a need for further selection for yield and stability and a better understanding of the metabolism. Thermophilic bacteria have frequently been proposed for the production of ethanol from lignocellulose. These organisms have a wide range of substrates, are capable of using pentose as well as hexose sugars and produce cellulase. One study suggests that ethanol made from wood using thermophiles can be half the cost of ethanol via cellulase and yeast. This would make the cost of the former equivalent to that of ethanol from maize.

Hydrogen

Hydrogen may be regarded as the ultimate clean transportation fuel. To produce it, water molecules have to be split by electrical or photochemical, includ-

ing biological, processes, and when burned, only water is produced. Taken to the limit, this represents direct conversion of solar energy into transportation fuel.

Micro-organisms capable of producing molecular hydrogen include both chemotrophs and phototrophs (organisms deriving their energy from chemical reactions and light respectively). Hydrogen-producing chemotrophs often exist in mixed cultures which also produce methane and their use will depend on the availability of cheap oxidisable organic substrates (*e.g.* wastes).

Hydrogen-producing anaerobes are normal constituents of the consortia of organisms which are involved in the anaerobic degradation of wastes to form methane. Ethanol and organic acids are also intermediates. It may be possible therefore to interrupt the process to yield either ethanol or hydrogen as the ultimate product. Some hydrogen-producing bacteria are able to grow on cheap carbohydrate substrates. Calculations show that the efficiency of energy conversion to hydrogen does not exceed 33 per cent of the combustible energy in the organic substrate. However, when the organic substrate is decomposed with the production of methane, the efficiency of energy conversion may be as high as 85 per cent.

More promising for hydrogen production are the phototrophs. Much attention has been paid to both purple bacteria and cyanobacteria but the latter are more attractive because hydrogen production is linked to the photolysis of water. Cyanobacteria are unique in their ability to produce hydrogen using water as their ultimate electron substrate, CO_2 and nitrogen from air, and solar energy as an energy source. A two-phase system is used whereby in the first phase the cells take up CO_2 photosynthetically, and in the second produce hydrogen using the photosynthetic products. Continuous production of hydrogen by immobilised cyanobacteria in a photobioreactor over a period of five months has been demonstrated on a laboratory scale.

Stationary power

Lignocellulose materials, which include wood produced by short rotation forestry or coppicing, waste material from conventional forestry, agricultural wastes such as straw and domestic organic refuse, and energy crops such as miscanthus, can all serve as fuel for combustion and gasification.

A biomass to electricity industry might develop initially on the basis of existing forestry and agricultural residues which are produced in vast quantities – more than 2 billion tonnes annually throughout the world. For example, studies of the residues from the sugar cane industry and the pulp and paper industry indicate a combined power export capability sufficient to meet 5 per cent of the current global electricity demand.

Short rotation energy forestry projects have examined the use of willows and poplars, in temperate regions, and eucalyptus and leucaena (an N-fixing tree legume) for the tropics. Projects have pointed to a need for new management techniques and matching species to location. Biomass from trees grown specifically for power generation would be diverted from the process of oxidation by decay, to oxidation within an energy recovery system – the flow of carbon back to the atmosphere remaining essentially unchanged.

Combustion technology for biomass based on fluidised beds was developed in the 1970s, and a gasifier on the same principle in the early 1980s. Pressurised fluidised bed combustion technology is under test as part of the US DOE Clean Coal Technology programme. Both low- and high-pressure gasifiers which will utilise biomass are being developed. In the context of modular biofuel plants, aeroengine-based gas turbines have possible advantages over their industrial equivalents, including efficiency, maintenance and development potential. A leading contender for combined operation is the BIG-GT (Biomass Integrated Gasification – Gas Turbine) cycle. A recent Shell study identified this technology as a leading option for biomass energy in the 21st century.

One of the major problems in the developing world is obtaining wood for cooking fires. Much deforestation and desertification is a consequence of this search. Where wood is not available or is too far away, vital natural fertilizers in the form of animal manure are used instead. To ensure a sustainable supply of firewood in third world countries, a proposed solution is to grow tree legumes (*e.g.* ipil-ipil and casuarina) which have N-fixing symbiotic micro-organisms. A small increase in the efficiency, currently 1-2 per cent, with which firewood is burned in developing countries, by using simple stoves rather than open fires for example, will have an immense effect on carbon emissions.

Biogas

Biogas usually consists of a mixture of methane, nitrogen, water vapour and carbon dioxide, with sulphur compounds, such as H_2S, as corrosive by-products. Methane from biological sources is generally produced on a scale more suitable for domestic or commercial consumption than for centralised power plants. It is usually used for heat production, although small generating sets have been designed.

Essentially three basic types of biogas production exist – from landfill; from dedicated sources of biomass; and as a by-product from anaerobic treatment processes for sewage sludge, domestic animal slurries and high-strength industrial waste streams in appropriately designed bioreactors. In the case of waste materials, it should be remembered that the primary process objective of such systems is effective waste treatment rather than efficient biogas production.

The formation of biogas represents an efficient method for recovering chemical energy from very wet organic waste. The chemical energy in sludge is just about enough to sustain combustion if the sludge is dewatered to 20-25 per cent solids. This is close to the upper limit for filters or centrifuges. As biogas, the fuel automatically separates from the liquid phase and can be burned as such in furnaces or modified internal combustion engines. If water vapour, H_2S and CO_2 are removed, the remaining methane, about 50 per cent in volume, can, after further purification, be compressed and shipped in natural gas pipelines.

Anaerobic digestion of wet biomass and wastes is increasingly being carried out in order to convert the organic content to usable methane. Digestion utilises a complex consortium of bacteria and archaebacteria, functioning at ambient pressures and at nearly neutral pH and converting biodegradable organic matter into methane and carbon dioxide in the absence of oxygen. Conventional temperatures for digestion (25-35°C) are not high enough to destroy pathogens. However, newer technologies involve thermophilic digestion at 55°C, and it is accepted that this temperature, held for 24 hours or more, will destroy most pathogenic organisms.

The relative importance of anaerobic digestion for waste control, versus the generation of methane as a fuel, varies in different locations and is dependent on the price and accessibility of alternative fuels, the ability of a population to buy that fuel and the availability of feedstock. In developed countries, disposal of waste may be more important, with gas generation a useful and significant by-product. In poor countries, with proportionately large agricultural sectors and high numbers of farm animals generating manure, extensive use is made of simple anaerobic fermenters in rural areas. In India, some 150-200 000 new waste digesters (village scale) are being built each year. The programme target number of 1.2 million will save 4.2 million tonnes of fuelwood and 20 million tonnes of manure. Community biogas plants provide fuel for electricity generation for domestic and irrigation water pumping, illumination and small rural industries. Design improvements in this appropriate technology (digestors and generation sets) are still required.

There is considerable scope for genetic improvement of anaerobic digestion, particularly in the area of organisms stable at higher temperatures resulting in higher digestion rates. Also, the process is exothermic and currently requires cooling. Additionally, higher temperatures mean better removal of pathogens. Anaerobic digestion will become more efficient and reliable through the development of methods to concentrate methanogenic biomass in the reactor and through the use of on-line control devices. The "missing link" is the technique to establish effective microbial associations – microbial ecology. Some of the most extensively studied consortia with respect to co-operative metabolisms are those involved in the anaerobic methanogenic biodegradation of sugars, carboxylic acids (other than acetate) and other soluble compounds to form methane. A

feature of these latter systems is the existence of two methane-producing steps, from acetate, and also by the reduction of carbon dioxide with hydrogen.

Wider application of biofuels will require R&D on the following topics:

- *Oil crops:* Increased yields of crop-plant oils with reduced fertiliser inputs; development of acceptable fuels and higher yields from a wider range of plants, especially trees [for example, yields using new palm clones (Unilever) have risen from 7 to 14 tonnes/ha].
- *Ethanol:* Improved processing of a wide range of biomass raw materials to ethanol – more efficient fermentation, including continuous processes and organisms tolerant to product concentration; novel enzymatic systems for lignocellulose breakdown.
- *Lignocellulose:* Improvement in gasification efficiency, selection of tree species and superior trees; choice of species (including N-fixing) suitable for third-world plantations.
- *Anaerobic digestion:* Identification of thermophiles, mechanisms within consortia, the microbiological physiology underlying better bioreactor control.

VI. AGRIPRODUCTION

Commercial agriculture and forestry represent both major sources and sinks for the three primary greenhouse gases: carbon dioxide, methane and nitrous oxide (N_2O). Conventional, high yield agriculture, requiring inputs of fossil fuel energy, and high levels of pesticides and fertilizers, is anything but "green" in the sustainable sense. There is as yet no clear understanding of how modification of agricultural practice will increase or decrease the relative emissions of these three gases.

A major contribution of biotechnology to agriproduction will be the use of genetic technology to improve the photosynthetic and reproductive capacity of biological material. New biotechnology can affect every stage of plant life: breeding, growth, harvesting and residue treatment; and at each stage there could be a consequent benefit for the environment in the form of more efficient, less resource-consuming, less polluting agricultural practices. Biotechnological developments are permitting the more rapid breeding of higher yielding plants having a higher proportion of harvestable material. Fundamental breakthroughs may come from new genetic modification methods that aim at increasing plant resistance to virus and other diseases, as well as tolerance to drought, salt, cold and heat. This will dramatically enlarge the land resource available for crop production.

Climate models indicate that a consequence of global warming will be greater fluctuations in rainfall and temperature with the consequent need to breed crop plants that can withstand these changes and still produce a reasonable yield. In the United States and Europe there are arguments for a shift from wheat to rye and sorghum ahead of global warming, while in developing countries where these crops do grow the shift is currently towards wheat – which is to their disadvantage as wheat is not appropriate to their climatic conditions. The benefits of biotechnology will not reach the developing world unless the genes and germ-plasm become available to them. OECD countries need to facilitate the transfer of know-how relating to the exploitation of plant biotechnology. Some genes, particularly those conferring resistance to disease, could have a major impact on third-world yields and on global pesticide consumption.

Any prognosis for plant biotechnology is complicated by the current long timescale for developments to reach practical agriculture – typically 20-30 years. Forecasts for developments over the next 20 years include genetic manipulation of many agronomically important traits but these will mainly be associated with single genes. Improvement through genetic engineering of traits controlled by several genes, such as tolerance to cold or drought, can be expected only much later. Very little is known as yet about the effect of introduced genes on plant metabolism. While improved yields are expected from better nutrient assimilation, they may also result from improvements in leaf area and duration.

New perspectives are being opened up by the development of a wider range of agricultural biotreatments. Bioinsecticides (*e.g. Bacillus thuringiensis*) have been known for some time, but new possibilities include seed treatments (inocula of growth-promoting pseudomonads), plant vaccines, bioherbicides and probiotics. The use of biocontrol agents to replace recalcitrant chemicals will reduce pollution and the need for conventional chemicals from fossil fuels.

Increasing crop yield requires more than just plant genetics – it also needs the application of water and fertilizer. Currently, millions of tonnes of excess fertilizer are applied, and there is a need for novel approaches to crop production, making use of all modern technologies. Replacement of nitrogen fertilizers with nitrogen fixation in or around the plant should therefore be another priority goal of plant genetics. Nitrogen is fixed and made available to certain plants (primarily the legumes) by symbiotic bacteria located in nodules on the plant roots. The mechanism by which this occurs has been a major target for plant researchers for many years. Improving the nitrogen-fixing efficiency of the symbiotic bacteria is the only likely improvement to reach the farm over the next 20 years, with priority crops being rice and wheat. Transfer of these genes from N-fixing to non-fixing organisms should also be achieved. It may be possible to introduce genes for nitrogen fixation into the plants themselves, but it should be borne in mind that N-fixation

requires considerable metabolic energy and is likely to result in decreases in yield.

Some plant species have different metabolic pathways for fixing CO_2. Most temperate crops and all trees have the so-called C3 metabolism, while most tropical and sub-tropical species such as sugar cane and maize utilise an alternative, C4, pathway. The latter are able to utilise low concentrations of CO_2 more efficiently and therefore tend to have higher biomass yields. Most plants, especially C3 plants, can exhibit 30-40 per cent higher productivities under elevated levels of CO_2. Transfer of carbon to roots and symbionts in the surrounding soil is favoured by CO_2 enrichment. It is generally agreed that the current CO_2 level in the atmosphere is sub-optimal for photosynthesis and dry matter yield. For their carbon supply, roots are dependent on transport from the shoot and they form a considerable sink. A major source of carbon loss occurs in C3 plants as a result of photorespiration in which O_2 is fixed and CO_2 respired. This loss can amount to 30 per cent of the C already fixed. In C4 plants this loss is negligible. So far, attempts to select C3 species with lower photorespiration have proved unsuccessful, but yield increases of 10-20 per cent as a result of photosynthetic improvement are forecast. The C4 photosynthetic mechanism equips plants with valuable competitive advantages under conditions of high light intensity, high temperature and low water availability. Their greater efficiency in trapping carbon dioxide means that they can exert a tighter control over water balance than most C3 plants. There is considerable debate as to which class will benefit from climate change and what the value might be of transferring the genes for efficient C metabolism to other plants.

The maximum efficiency by which plants may convert and store the energy of natural sunlight is perhaps 12 per cent. A large initial loss in efficiency occurs because less than 45 per cent of the spectrum is photosynthetically active. Quantum effects within the photosynthetic mechanism, plant respiration, leaf reflection and leaf area, all decrease efficiency even further. In general, an overall annual solar capture efficiency of about 1 per cent is normal, and this may reach 2.5 per cent in exceptional field conditions with C4 plants. Algae and phytoplankton, on the other hand, may reach levels as high as 5 per cent.

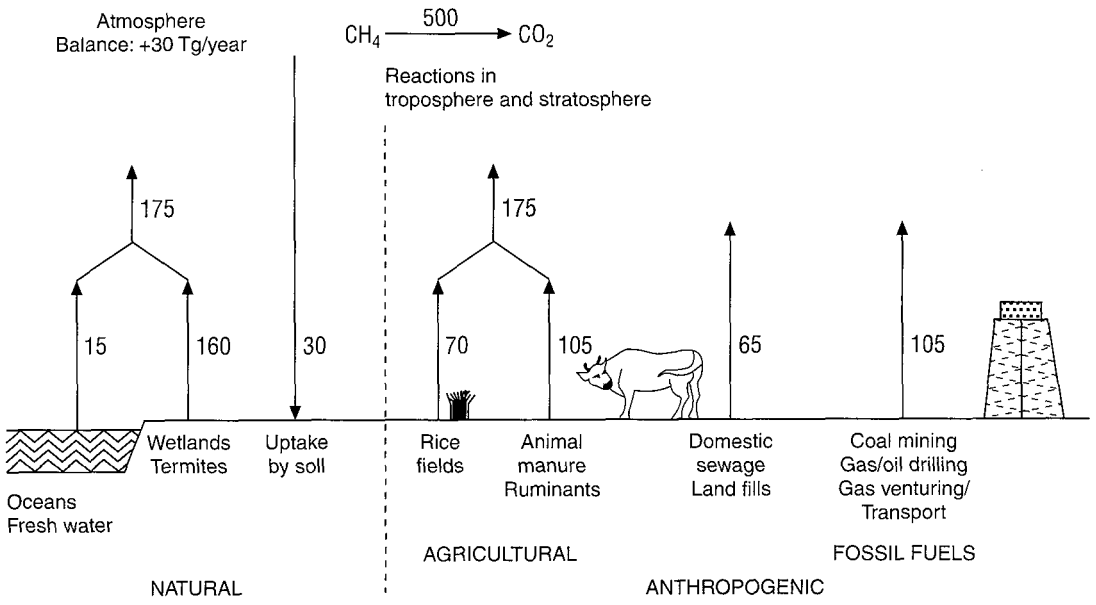
In the very long term it may be possible to reduce photorespiration with the aid of genetic modification of the major photosynthetic enzyme system – ribulose 1.5-bis-phosphate decarboxylase/oxygenase (commonly known as "Rubisco"). The properties of this enzyme vary considerably from species to species with, for example, plant enzymes having a ten-fold higher affinity for CO_2 over the algal enzyme but only a third of the turnover. Studies currently being undertaken may lead to modified enzymes with improved performance. The photochemical reaction within the plant is, however, very efficient (90+ per cent) and is unlikely to be

improved by any genetic engineering. In trying to increase yield one always needs to consider the implications for the physiology and architecture of the whole plant.

Methane emissions

Paddy fields are a major global emitter of methane, with the quantity of methane emitted depending on soil factors, nutrient management, water regimes and cultural practices (Figure 3). Paddy field ecosystems are too complex, however, to justify attempts to modify them, by adding "foreign" organisms for example, until a better understanding exists of organism integration. Better agricultural management to reduce methane emission is, on the other hand, a possible route to methane reduction. For example, addition of sulphate to soil reduces methane by raising the redox potential and the activities of sulphate-reducing bacteria which out-perform the methanogens. Addition of nitrate has a similar effect. However, the greater potency of N_2O as a greenhouse gas, compared to methane, means that no risk of increasing its emission must be taken just to reduce methane emissions. The methane production rate is dependent on fertilization of the paddies, more organic nutrients being correlated with a higher methane emis-

Figure 3. **Simplified scheme of important methane sources and sinks**



Source: IEA/OECD (1994), *Scoping Study: Energy and Environmental Technologies to Respond to Global Climate Change Concerns*, Paris.

sion. This means that better control of fertility may reduce methane production. Also, control of the water level with intermittent flooding may be one route to reducing methane emissions but this again may encourage N_2O emissions. Since dry rice cultivation results in much lower emissions of methane, a shift from wet to dry cultivation of rice will reduce global methane emission, but such a change has enormous social implications. The yield of rice in paddies is presently much higher than that of dry fields and selection of rice varieties that show a fast growth and a high yield on relatively dry land will be essential. Different rice cultivars have different capacities for methane emission, which provides an option for improvement using conventional breeding techniques or genetic engineering. The importance of genetics in future rice cultivation is stressed by the Chinese decision to spend US$3.8 million to elucidate the base sequences of rice DNA.

Most ruminants (cattle, etc.) are another important source of methane as a consequence of the breakdown of foodstuffs by micro-organisms in the intestine. Organic materials in feed are fermented into organic acids, etc., and at the same time H_2 and CO_2 are produced. These are further converted to methane or to acetic acid. The methane, which represents about 10 per cent of the digestible energy consumed, is emitted into the atmosphere. Research is under way to promote acetic acid production (which has a nutrition value) and to suppress methane formation. Intermediate in the production of methane in the cow rumen is the production of hydrogen. Up to 80 litres of hydrogen may be formed daily in the rumen as a result of fermentation of plant constituents. There is, however, no accumulation of hydrogen since it is consumed by the methanogenic bacteria present.

Present cost-effective methane-reducing options in ruminants include improved nutrition, physical and chemical feed processing, strategic diet supplementation and improved reproduction (twinning, embryo transplantation, etc.). Of these options, the most influential are feed characteristics and composition. Enzymes are presently incorporated into animal feeds to increase the availability to the animal of dietary minerals while at the same time reducing the nutrient content of their waste. Addition of enzymes may also aid digestion and thus reduce methane production. Dietary conversion may also be improved by pre-treatment of the feed (pre-digestion using silage additives).

There may be over 200 species of organisms in the rumen. Although this is a complex ecosystem, dietary supplementation with live bacteria (probiotics) is practised, although not widely. Probiotics for methane reduction should promote the conversion of hydrogen and CO_2 into acetic acid and inhibit the methanogenic bacteria. This modification saves the environment in two ways: prevention of methane emission and better feed efficiency. It is conceivably possible to replace organisms in the cow's gut with genetically modified organisms making better use of foodstuffs, but this is such a complex ecosystem that it is very uncertain what

might be the effect. More insight and understanding is needed to replace the trial and error approaches adopted so far.

Desertification and salination

Widespread desertification, like deforestation, is a major source of atmospheric CO_2. A long-term priority is the breeding of plants, such as xerophytes and salt-proof plants, compatible with desert conditions, using gene recombination and cell fusion techniques. Plants capable of surviving in dry conditions do so by suppressing water loss by reducing the stomatal apertures in their leaves and by developing waxy coatings. CO_2 uptake however requires the stomata to be open. A target of research, in addition to a more efficient Rubisco, is a better understanding of the genes controlling water retention.

A route to sequestering carbon is to grow more biomass, such as new forest, over a larger area of the Earth's surface. This means bringing into cultivation marginal lands – including areas affected by salt (estuaries, etc.), and drought (deserts). The applications of biotechnology for reversing desertification may be divided into short- and medium-term technological developments, and long-term basic studies. The main short-term issues include water retention and the prevention of salt damage. A research group in Japan has developed a new "super-bioabsorbent" which can be incorporated into soils and which will absorb and hold water at more than a thousand times its own weight. Casuarina, a tree legume, grows well in dry and salty conditions, and is currently being tested in Egypt and Senegal.

Mangroves, which grow in salt water, are a primary agent for reclaiming land from the sea. These, and also the macroalgae, sea-grasses, etc., may be a source of genetic material for faster growing crop plants, permitting them to grow in brackish soils (and where irrigation has brought salt to the surface). The result could be halophyte crops, genetically engineered, which would thrive under sea-water irrigation. There is thus a four-fold target for R&D: an increase in productive land area; improvement in water usage; higher yields of food and renewable biomass; and the sequestering of atmospheric CO_2.

Genetic engineering – the new biotechnology – will change every aspect of a plant's life, from breeding and growth to harvest and residue. Each improvement can be environmentally beneficial – every increase in yield and reduction in fertilizer use will ameliorate global warming. Every move in the direction of disease resistance, drought and salt tolerance means an enlargement of land resource and CO_2 sink area.

Atmospheric CO_2

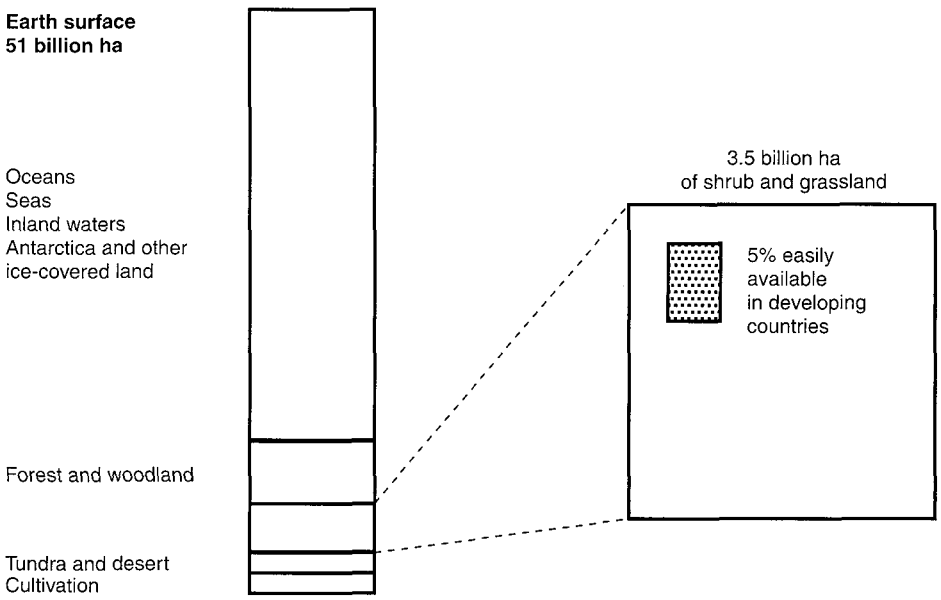
Carbon fluxes related to photosynthesis and respiration are an order of magnitude larger than those from fossil fuel combustion (see Figure 1) and indicate the possibilities for beneficial change. The wide range of estimates of deforestation and land use flux, estimated at 0.6-2.5 Gt C/annum, reflects the fact that terrestrial vegetation and soils are extremely heterogeneous and that it is difficult to assess changes in carbon storage per unit area cleared and the effect of regrowth of abandoned land. Important uncertainties exist about the land biota (particularly concerning growth stimulation by anthropogenic CO_2 and nitrogen compounds), reaction of photosynthesis and respiration to temperature and water cycle changes and longer-term modifications of ecosystem composition due to environmental change. Investigations of the long-term change in carbon uptake by the biosphere must also take into account how vegetation patterns will change in response to climate and decisions on land use. Until such an analysis is possible, predictions of future CO_2 concentrations and the efficiency of policies directed at stabilising atmospheric CO_2 must remain questionable.

Energy forestry, producing wood to substitute for coal, would result in CO_2 emission reductions of 17 420 kg per hectare per year. This may be compared with about 5 000 kg per hectare for ethanol from sugar beet. A Shell study calculated the subsidy required for short rotation forestry at about US$360 per hectare, compared with US$1 050 to US$1 805 for liquid biofuel options. Recently, interest has increased in growing energy crops on agricultural land taken out of production ("set aside" land) in Europe and the United States.

Ignoring the vast scale of the undertaking and a range of complex issues that have yet to be researched adequately, such as land availability and local socio-economic and environmental impacts, a planted area of around 100 million hectares or 2.5 per cent of the existing forest area has the potential to offset one-quarter of current atmospheric CO_2 accumulation for an unlimited period. Globally, I00 million hectares of under-utilised land (14 million in the EU, 60 million in the United States) would be relatively easy to find (Figure 4) and a reasonably productive, distributed energy forest of this size, coupled with BIG-GT technology, would be capable of supplying one-third of current global electricity demand.

Dissolved CO_2 is consumed in the mixed layer at the ocean surface by photosynthesis. Some of this assimilated carbon sinks as organic debris to the deep-ocean, where it is largely mineralised to inorganic carbon. The marine biota are thus pumping carbon to deeper layers in the ocean. The time needed for carbon to return from below the thermocline (topmost 1 000 m) is several decades, and many centuries are needed for recycling carbon from the deep-ocean reservoirs.

Figure 4. **Scope for afforestation**



Source: Author.

It may be possible to encourage the growth of plankton at sea. CO_2 built into their carbonate skeletons will, on the death of the organisms, sink to form stable sediments on the ocean floor. A conservative estimate indicates that CO_2 usage could easily be expanded by a factor of ten or more if new or improved processes could be developed. Biologically, CO_2 has been used to produce fats from algae for biodiesel fuels and the antioxidant vitamin β-carotene. Though algae-derived biodiesel has only been produced in laboratory-scale quantities and β-carotene production world-wide is only 10 tonnes/annum, such biological systems, if developed, have the potential to produce many different oxygenated chemicals from CO_2 in addition to biodiesel.

Priority areas for agricultural and forestry R&D will include:

- *Agriculture:* Development of disease-resistant, faster growing and generally novel plants and trees; improved photosynthesis, biofertilisation (nitrogen fixation); better understanding of plant genetics especially in areas of drought resistance and salt tolerance; improved carbon-fixing enzymes (Rubisco).
- *Animal husbandry:* Studies of rumen digestion and ecosystems; probiotics; feed pre-treatments.

- *Forestry:* Research into superior trees (sustainable forestry) for energy production, timber and paper; improvement of tree legumes for third-world forestry; development of the ability to transform tree cells and regenerate whole trees from these.
- *Atmosphere-related:* Interactions within ecosystems such as rice paddies; understanding of biological sources and sinks for CO_2, methane, nitrous oxide and the effect on these of human intervention.

VII. MEDIUM- AND LONG-TERM OPTIONS – BIOTECHNOLOGY IN THE FUTURE

"I do this with some trepidation. We've all smiled at predictions from the past that look silly today", Bill Gates, The Road Ahead.

The acceleration of understanding of the underlying principles of biotechnology – the structure and function of the gene and how it is reflected in the proteins, both structural and catalytic, that it determines – over the last 20 or so years has been astounding. Technologies such as DNA cloning, sequencing and synthesis were unimaginable a few decades ago. Significant advances in genetic mapping techniques have been made for example as a result of the Human Genome Project. This rate of change shows no signs of letting up – applications of these techniques have the potential to transform the quality of life. Consequently, it is not too far-fetched to say that in another 50 years of biotechnology, the determining rate of new developments will be not what the technology can offer but what mankind may wish.

Genetic modification

Just as chemists have circumvented natural product formation in producing xenobiotics, molecular biologists are able to use recombinant DNA (r-DNA) technology to form organisms with novel synthetic and degradative capacities. Genetic recombination involves a restructuring of DNA molecules so that new genomes are formed containing information from different DNA sources. Genetically modified organisms (GMOs) are already being patented. An early use of pure cultures of selected or engineered micro-organisms will be to abate pollution. Industrial effluents are particularly suitable for directed degradation because the starting material is reasonably well-defined. Contained use in bioreactors of GMOs selected for particular recalcitrant molecules will be the first applications.

It is likely that GMOs will ultimately be used *in situ:* laboratory research and novel detection capabilities are going hand in hand to make this possible with little risk. However, regulatory problems associated with the release of genetically

engineered organisms may militate against their early use and, since it will be possible to isolate a much wider variety of natural organisms, it is possible that the selection and tailoring of naturally occurring organisms and the use of GMOs will go together.

The production of purified enzymes has become more efficient through r-DNA techniques. These developments improve the chances for more biotechnologically-based production processes for a wide range of chemicals. In addition, the development of engineered enzymes may lead to spectacular process advances. So-called synzymes, synthetic biocatalysts, synthesised from their amino acid building blocks, and capable of functioning under a diverse range of temperatures, pressures and solvents, will be the basis for a wide range of biosynthetic processes.

In nature, pure cultures do not exist and all biotechnological reactions are carried out by communities or consortia of organisms. In laboratory experiments with very limited consortia (perhaps two or three species) it has often been the case that yields or conversion rates have been considerably enhanced. While our understanding of the interactions between organisms in these circumstances is very limited, it is, nonetheless conceivable that, in the first decades of the next century, sophisticated consortia of micro-organisms will be used to carry out many production processes and that the ultimate carbon source, CO_2, will be a widely used raw material. There is a need to characterise and identify organisms within ecosystems. This understanding will in turn enable practical use to be made of unique adaptations and allow the management of ecosystems.

The same may be true for the development of new ecological systems. At present, the introduction of a plant or micro-organism into the natural environment often fails due to lack of adaptive capacity to such a complex system. As this is a prerequisite for (re-)production and proliferation, considerable study is essential in order to unravel the ecological interactions.

Food, agriculture and forestry

It is possible to imagine foods derived from fungi or algae having almost any texture and flavour – some are already being consumer-tested and marketed. This may reduce the need to grow animals as a food source. Not only will this make more cereals available to feed the considerably increased population, but fewer ruminants means less ruminant-derived methane.

The complete sequencing of the human genome will be shortly followed by a full knowledge of plant gene sequences. This in turn will revolutionise yield, introduce effective N-fixation, etc., and genetically engineered halophytes.

Fuller attention to the relationship between wood structure while growing and the physical properties required of constructional and other materials could mean the possibility of genetically engineering trees to meet a much wider requirement, replacing components made from fossil fuels and at the same time effectively sequestering carbon. An ultimate goal might then be to improve the functionality of natural polymers within the timber itself.

The marine biosphere

While the oceans cover the major part of the earth's surface, the processes that go on within them, be they physical or biological, are among the least understood. Thirty per cent of the net global biomass production is thought to occur in the oceans but the relationship with atmospheric CO_2 is very uncertain.

Identification of marine micro-organisms is even less adequate than of land-based organisms. Nonetheless marine biotechnology is regarded as the greatest untouched resource of novel processes. There is much fundamental research to be done, including the identification of U.V. tolerant forms of marine organisms, ecologically important forms of phyto- and zoo-plankton and the strange recently discovered barophilic organisms of the hydrothermal vents.

Artificial photosynthesis

For many years it has been the goal of photochemists to provide an artificial system which mimics the unique properties of photosynthesis, particularly the ability to achieve a very rapid and efficient separation of charge without a significant back-reaction. If this could be achieved, it would be possible to use solar energy to produce electricity directly, to split water, or to fix atmospheric CO_2 into useful molecules. Such systems require catalysts which reproduce the photosynthetic mechanisms of plants. These may be based on organometallic complexes or, alternatively, light harvesting chlorophyll and protein complexes can be reconstituted onto artificial membranes. These should be capable of collecting energy and transferring electrons with efficiencies comparable to photovoltaic cells. It is expected that highly efficient algal photolysis of water should be practical by the year 2010. However, considerable advances in chemistry are required to develop artificial photosynthesis systems and it is unlikely that this will be possible on any scale before mid-century.

Hydrogenases from a number of phototrophic organisms have been used as part of laboratory systems for the photocatalytic production of hydrogen and are considered as important future industrial components because of their high specific activity and because the resources for their production are virtually unlimited. However, their present stability compares unfavourably with chemical catalysts

and research is being directed towards an improvement. In the very long term, this may lead to solar-driven biofuel cells for electricity generation.

Achievements in the next three decades

On the assumption that industrial companies and national governments are prepared to support this level of effort, it is possible to speculate on what may be achieved in the coming decades. Every reader of this article will have his or her list of successes and failures; the following is that of the authors alone:

Bioremediation

- modular, high throughput, combined aerobic/anaerobic treatment processes will be available for both domestic and industrial waste water streams;
- a high proportion of contamination in soil will be treated *in situ* using combined physico-chemical and biological techniques;
- sensors based on biological principles will be used to monitor ecotoxicity both in water and contaminated soils;
- advanced bioprocesses will be used to treat a wide range of volatile pollutants in agricultural and industrial off-gases.

Biocatalysts

- a wide range of chemical processes, in addition to those used in the manufacture of pharmaceuticals, will include biological steps;
- genes for extremozymes will be expressed in common organisms for manufacture in quantity;
- several synzymes will be in use.

Novel and renewable materials

- more than one biodegradable plastic will be on the market;
- lignocellulose will be a common raw material for chemical processing;
- genes for a number of high value chemicals will be expressed in commonly grown plants.

Biofuels

- continuous, high temperature fermentation under vacuum of both starch and cellulose for ethanol production will be economically competitive;

- economic production of palm oil esters; 50-100 per cent yield increase of oils from crops such as rapeseed;
- managed low input forestry plantations yield 25-35 tonnes DM/ha/a in temperate and 45-50 tonnes DM/ha/a in tropical areas;
- the development of a high rate, high temperature, modular anaerobic digestion process, applicable to most domestic and agricultural wet wastes obviates the use of landfill for any organic materials;
- significant yields of hydrogen from algae and the first stages of photosynthesis improvement.

Agriculture and forestry

- 20 per cent increase in general crop yields, genetically incorporated disease resistance in a number of economic crops; later, most pest treatment and fertilisation achieved biologically;
- economic benefit from probiotics in cattle; widespread use of enzymes for improved feed usage;
- sustainable third-world firewood plantations and tree species bred for modified composition of wood and reduced emissions of volatile organic compounds;
- low emission agricultural systems understood and in place.

VIII. CONCLUSIONS

This article has tried to illustrate a variety of ways in which biotechnological products and processes fit under the heading of "green technology". The new biotechnology – genetic engineering – will make possible a far wider range of possibilities for producing environmentally friendly materials using technologies that generate little or no waste. However, environmental biotechnology products cannot succeed on their own; they must be integrated with existing technologies in a holistic approach to care for the environment. Microbial pesticides, for example, will become a useful component of integrated pest management, but should not become its replacement. Generally speaking, biotechnology can provide optional solutions, means and modules to be applied in conjunction with other technologies.

The future use of biodegradable plastics, as of other renewable materials, will depend on a balance of economic factors and societal needs and values. Without a doubt, the costs for biomaterials will be higher than for the fossil-fuel-based materials they may substitute. Successful commercialisation of PHA polymer production, for example, will depend on an increase in product yield, reduction in

capital costs of the technology, and improving recovery of the polymer from the rest of the cell biomass.

Many environmental groups maintain that the best approach is for consumers to substitute other materials entirely where possible, using paper packaging, for example. Recycling may make a bigger contribution to solving the landfill crisis. Waste plastics need not always be separated before recycling: "co-mingled" plastics can be turned into useful construction materials. Ironically, if degradable plastics become very common, they could eventually create a problem by weakening recycled plastic.

Natural and global systems are so complicated that long before we rush in to apply a new technology, we need to study very carefully all the ramifications. The Toronto conference of 1988, in discussing global warming, concluded that: "Humanity is conducting an enormous, unintended, global experiment... with unknown consequences". It may be appropriate to say that some of the remedies proposed are in the nature of similar experiments. Although there are indeed actions that may be taken now, future technological fixes may pose unforeseen risks unless fundamental research is carried out beforehand.

From within the subject areas covered above, it is possible to identify many targets for future research. Much progress has been made in the application of biotechnology as a result of pragmatic experimentation. There is great potential but the new developments foreseen will require considerable scientific effort – the opening of the "black box". While a range of short-term developments are possible based on existing knowledge, the longer-term contributions that biotechnology can make to a better environment require fundamental scientific programmes and the development of new applications in addition to appropriate policy frameworks. Some of this R&D may appropriately be local or national but the scope of much of the work is such that it will require international co-operation.

Biotechnology will give rise to sustainable solutions, but we will need to be fully aware of the social, legal and economic changes that will affect the technology and to which it will give rise.

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BIOTECHNOLOGY AND WATER CONSERVATION

TABLE OF CONTENTS

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I. INTRODUCTION

This epoch will be remembered as the one in which human kind began to be aware of the limitations of the world's natural resources. Accomplishing a wise balance between nature's limitations and the exploitation of the environment by man is a challenge that we cannot afford to postpone. Otherwise, the future of the Earth's resources, and human life itself, will be put at extreme risk.

One of the most important natural resources which is present in limited supply and threatened by defective management is water. Water is a fundamental support of ecosystems, and is vital, ubiquitous, badly distributed and scarce. During human history water has been a key factor of patterns of population growth, economic development, social-political organisation and, in general, of the quality of life. Water is a cause of war and a catalyst for peace (Naff, 1993).

Seventy per cent of the globe's surface is covered by a combination of saline and fresh water, with a total volume of 1.41 billion km^3. However, 98 per cent of this water is saline or otherwise unusable for human and animal consumption. Only 2 per cent of the total volume of Earth's water is fresh, and two-thirds of that supply is frozen (the polar and glacier zones), leaving 1 per cent as liquid, consumable fresh water. These freshwater resources are unequally distributed among the continents and within them (Naff, 1993). South America has the most abundant runoff (26 per cent of the world total) of any continent, followed by Asia (25 per cent), North America (17 per cent), the former Soviet Union (12 per cent), Africa (9 per cent); and Europe (6 per cent), while Australia and New Zealand/ Oceania (5 per cent) have the least runoff (Chapman, 1990).

An important characteristic of water is its variability over time. For example, seasonal fluctuation of river runoff can be extreme and becomes a factor influencing utilisation strategies. More regular utilisation and distribution can sometimes be created with water storage basins. There are some 35 000 high dams in the world and 100 new high dams are constructed annually (Chapman, 1990).

At present, almost 3 500 km^3 of freshwater are withdrawn annually, almost twice the store of freshwater in the rivers at any given instant. From this water, 40 per cent returns to rivers and other water sources as waste water, frequently in a polluted condition (Chapman, 1990).

Two interrelated world trends greatly exacerbate the water supply situation: population growth and economic development, both of which greatly increase water demand (Frey, 1993). Given these facts, more ways should be developed to control effluent use, and to practice more rational, co-ordinated, basin-wide water management; otherwise, the region's water consumers face a certain future of increasingly severe water deficits.

One clear example of water mismanagement is found in agricultural use. Averaged globally, 73 per cent of the water withdrawn from Earth goes to agriculture (2 555 km^3). Almost 300 million hectares in the world are irrigated land and more is being added at the rate of 8 per cent a year. Irrigation, as it is practised in most countries, is highly inefficient. Averaged over the world, only 37 per cent of all irrigation water is taken up by crops, causing an enormous waste of the resource (Maurits la Rivière, 1989).

Another problem brought on by over-irrigation is salinisation. As water evaporates or is taken up by plants, salt is left behind. If the rate of salt deposition exceeds the rate at which the salt can be removed by flowing water, a salinisation problem can occur. It has been estimated that, of the total agricultural land available on Earth (14 billion hectares), nearly 1 billion hectares (7 per cent) are affected by excess salt, and 77 million hectares, one-third of the total irrigated land (230 million hectares), is believed to have salinisation problems (Ashraf, 1994; Chapman, 1990).

In addition, desertification is another challenge. Dryland is expanding at a rate of 6 million hectares per year (UNEP/GRID, 1996). This spread of desert-like conditions decreases biological productivity and contributes to loss of biodiversity, loss of biomass and to global climate change by decreasing evaporation and the loss of a carbon "sink" (UNEP/GRID, 1996). Desertification is very often a consequence of human activity (*i.e.* cultivation of marginal lands, deforestation, overgrazing, cattle raising, incorrect irrigation practices) (The World Wide Fund for Nature, 1996). In some cases, degraded land becomes agriculturally worthless. Land reduced to zero or negative net economic productivity is increasing at a rate of 21 million hectares annually (UNEP/GRID, 1996).

Among the 100 countries affected by moderate to very severe desertification, 90 are developing countries. Desertification threatens the health and nutrition status of over 900 million people, mainly poor (Dumont, 1996). Additionally, the cost of anti-desertification measures escalates from year to year, because the area affected, the degree of the damage and world prices of rehabilitative measures are also increasing (UNEP/GRID, 1996).

Assuring an adequate water supply and management is not the only water problem facing many countries throughout the world: they are also concerned about water quality. Freshwater pollution arising from human activities began with

the first human settlements and has increased in severity as populations have grown. Initially, pollution from organic wastes and salinisation were the major problems. To these must now be added the problem of waste generated by a wide range of industrial processes and by land use runoff (suspended solids, heavy metals, radioactive wastes, nitrates and organic micropollutants). Other pollution sources of growing importance are leaching from mine tailings, solid waste dumps and the atmospheric deposition of pollutants into water bodies (Chapman, 1990). Additional environmental problems such as the acidification of lakes and streams and the eutrophication of lakes and coastal waters have been generated.

Although organic waste is fully biodegradable, it presents a significant problem – and in some places (such as big cities) a massive one. Excessive biodegradation can cause oxygen depletion in lakes and rivers. Also, human excreta contain dangerous biological contaminants, including such pathogens as the waterborne agents of cholera, typhoid fever and dysentery, and a number of disease-causing viruses (Maurits la Rivière, 1989).

Another important issue involving water management and public health is the use of industrial waste water and municipal sewage for land farming. Waste water is used to irrigate crops world-wide: 500 000 hectares of cropland in some 15 countries (0.2 per cent of the world's irrigated area), and creates a significant public health problem because of the risk of contamination with pathogens (Verstraete, 1996).

In the last decades, purifying technologies have been a successful short-term measure to confront the water quality problem. Advances in new biotechnological methods (bioremediation) offer an important alternative because they are economically competitive and less harmful to the environment than traditional physico-chemical technologies.

However, it has become apparent that the prevention of pollution and the restoration of bodies of polluted water should gradually take precedence over the exclusive development of purification technologies. Water purifying technology could become more complex and costly as the number of pollutants present in water increases. The high cost of restoring polluted water bodies also strengthens the appeal of pollution-prevention programmes (Maurits la Rivière, 1989).

There are many ways in which biotechnology can help to prevent water pollution and to reduce water use. A long-term water conservation programme based on biotechnology could, in the first place, optimise existing technologies. For example, some conventional catalysts can be substituted by enzymes or the product yield of fermentation can be increased by the use of improved processes and micro-organisms, both resulting in reduction of water consumption. Secondly, biotechnology offers new alternatives (present and future), such as the use of

enzymatic processes in industry; genetically engineered transgenic plants resistant to salt, drought or pests; the use of biopolymers as new materials which can substitute some hazardous chemical products. These all fit the concept of clean technologies that are less resource consuming and are also environmentally friendly.

II. PREVENTION OF WATER POLLUTION

Bioprevention focuses on two main issues: preventing pollutant release to the environment; and creating alternative clean technologies (OECD, 1994). There are two ways to prevent release of pollutants: by the use of added-value processes – in which waste stream is converted into useful products; or by using "end of pipe" processes – where the waste stream is purified.

Clean technology includes the novel concepts of "green products" and "green technologies" – which have less impact on the environment while also being economically competitive with traditional technologies. Clean technologies are more efficient manufacturing processes, reducing energy and materials consumed and improving their recovery during waste management.

Industrial water management

Cleaner technologies

A good example of a novel clean technology is the use of enzymes. These proteins work as catalysts. Enzymes are biodegradable and can reduce water and energy consumption and the requirement for harsher chemicals, *e.g.* in bleaching processes. Some industrial uses of enzymes include (Stentebjerg-Olesen, 1996): leather industry and textile processing – where chemicals are replaced; paper industry; animal feeds – where enzymes upgrade feed; alcohol and beer production – where malt is replaced; and the use of enzymes as detergents.

Some other biotechnological innovations substitute for common processes and help to reduce environmental impact. Two examples are biodesulphurisation instead of hydrodesulfurisation for petroleum distillates; and the biological production of H_2 replacing fuel-based processes (Stentebjerg-Olesen, 1996). Bacteria of the genus *Rhodococcus* appear to possess the necessary metabolic capability to remove sulphur from sulphur heterocycles in petroleum. The genes for the process have been cloned and are thus amenable to genetic manipulation (Kulpa, 1996).

Hydrogen is considered to have a great potential as a future energy source because it is easily converted to electricity and burns cleanly. However, it is currently produced by fossil-fuel-based processes, which emit large amounts of CO_2 and other air pollutants such as sulphur dioxide (SO_2) and nitrogen oxides (NO_x). Biological H_2 production has thus recently received renewed attention owing to urban air pollution and concerns about global warming. The processes investigated most extensively are based on nitrogenase-mediated H_2 production by cyanobacteria (blue-green algae) and photosynthetic bacteria. In addition, microscopic green algae have been investigated as H_2 producers under light or dark conditions (Böguer, 1996).

New biomaterials

Another bioprevention approach is the development of new biomaterials; two important examples in this area are biopolymers and bioplastics.

Flocculants (synthetic high-polymers) are widely used in primary treatment of waste water. However, they have a great environmental impact due to the their toxicity. Biopolymers, such as protein bioflocculants and polysaccharide bioabsorbents, may be expected to overcome the problems associated with these conventional synthetic high polymers because they are biodegradable (Kurane, 1996).

The strains of *Rhodococcus nocardia* and *Corynebacterium* produce biopolymers that flocculate kaolin clay. For example, products from *Rhodococcus erythropolis,* which have a wide flocculating activity against both organic and inorganic materials, have been used for primary treatment of pig urine and excrement waste water. Also, a new super-polysaccharide bioabsorbent has been obtained from *Alcaligenes latus.* It can absorb water at more than 1 000 times its own weight, approximately five times more than currently used commercially available synthetic high polymer absorbents (Kurane, 1996).

Microbial polyesters are a typical biodegradable plastic. Several types of copolyesters of hydroxyalkanoic acids are produced by many bacteria from various renewable carbon substrates, such as saccharides and organic acids. Microbial polyesters are biodegradable thermoplastics whose physical properties can be regulated by varying the composition of the copolymer (Doi, 1996).

The bacterial polyhydroxyalkanoate (PHA) polymers have attracted much attention as environmentally degradable thermoplastics for a wide range of agricultural, marine and medical applications. PHA is degraded in soil, sludge or sea water. Some micro-organisms, such as bacteria and fungi, secrete extracellular PHA depolymerases to degrade environmental PHA and use the decomposed compounds as nutrients (Doi, 1996).

End-of-pipe processes

In Japan, carbon dioxide fixation with a microalgae end-of-pipe process has been studied. In nature, photosynthesis is the main process by which CO_2 fixation takes place; it is carried out by higher plants and algae. Biological CO_2 fixation by photosynthesis can decrease the concentration of CO_2 in the atmosphere, and the possibility of using it to reduce anthropogenic CO_2 emissions has been investigated (Matsunaga, 1996).

A biotechnological approach to eliminate CS_2 and H_2S from exhaust gas in cellophane manufacture has been proved to be a good alternative to conventional chemical treatment. It allows for achieving high removal rates from a flow with strong fluctuations in CS_2 and H_2S concentrations and temperature. The system described has the advantages of biological air pollution control equipment in terms of economy and harmless end-products (Revah, 1996).

Added-value processes

Two examples of using added-value processes are biogas production, from liquid squeezed from pineapple peel and core using a filter bed reactor and a fixed film digester (Warut *et al.,* 1986), and the synthesis of catechol from phenolic waste streams, using a strain of the thermophilic bacterium *Bacillus stearothermophilus* (OECD, 1994).

An evaluation of the possibility of the re-use of industrial orange wastes as organic soil fertilizer has been carried out. The pulp and peel orange wastes from an orange-juice industry were tested as fertilizers in lettuce cultivation, with satisfactory results (Correia Guerrero *et al.,* 1995). These kinds of use also work as added-value bioprevention processes.

Agricultural water use

It is well known that some chemical compounds used for agricultural purposes, such as fertilizers and pesticides, are among the most harmful water pollutants. Biotechnology offers an alternative, such as the use of biofertilizers and biopesticides instead of contaminating chemicals (OECD, 1995).

Biofertilizers

Examples of biofertilizers are the use of mycorrhiza, a symbiotic association between a fungus (from the Greek – *mykes*) and the roots of plants (from the Greek – *rhiza*) (De la Cruz *et al.,* 1991). The fungus utilises plant photosynthates as a carbon source, and the plant benefits from improved water regulation and mineral nutrient dynamics (see below). Vesicular-arbuscular (VA) mycorrhizal inoculants have mainly been used on a small scale on marginal soils such as acid

upland pastures, open cast mine reclamation sites, and semi-arid environments. Also there has been some recent success in developing commercial inoculants of mixed strains, mainly for use on high-value ornamental and vegetable crops, particularly in Japan (OECD, 1995).

Phosphorus, as an essential plant nutrient, limits crop growth when it is unavailable in a soluble form. To reduce phosphorus limitation, phosphate solubilisation can be achieved using several bacteria, fungi and actinomycetes. A widely studied fungus in North America is a strain of *Penicillium bilaii*. The fungus acts by colonising target crop roots and then secreting organic acids near the rhizosphere, therefore making possible solubilisation and uptake of normally insoluble inorganic phosphate compounds (OECD, 1995).

Cyanobacteria (blue green algae) have the capacity to photosynthesise and fix atmospheric nitrogen. This characteristic trait turns them into potentially promising biofertilizers. The most important blue-greens for this purpose are the filamentous genera possessing specialised sites of nitrogenase activity (the genera *Anabaena, Nostoc* and *Sesbania*). These micro-organisms are used as biofertilizers in South-East Asia and India (OECD, 1995).

Another example is the use of biomass as an excellent soil conditioner. In Germany, the biomass produced in the manufacture of the antibiotic cephalosporin C by the fungus *Acremonium chrysogenum* is separated using a filter aid (silica), and the filter cake contains 15-20 per cent silica. After the product is stabilised (adding 10 per cent of CaO), it can be supplied as soil conditioner (Crueger, 1996).

Biopesticides

Living cells for pest control offer another example of new bioproducts. The most important and most commercial biopesticides are based on *Bacillus thuringiensis* (commonly known as Bt) products. More than 50 different bug killing Bt proteins have been found among the thousands of strains of the bacterium (Strauss, 1996). Bt has been the active ingredient in a wide array of biological insecticides for nearly a half century. These products have been utilised as highly safe alternatives and supplements to chemical insecticides for applications in agriculture and forestry, and for the control of disease vectors such as mosquitoes, beetles, ants, mites, caterpillars and blackflies (Carlton, 1996; Strauss, 1996). In agriculture, most of the products have been based on a single strain of Bt, termed HD-1 (Carlton, 1996).

Since the 1980s, research has been undertaken on the development of new recombinant strains of Bt. The insecticidal crystal proteins (ICPs) produced by Bt strains are found in sporulating cells encoded on extrachromosomal plasmids. These plasmids are capable of being transferred between strains of Bt by a

microbial conjugation-like process. To date some 30 or more different ICP genes have been cloned and sequenced. The company Ecogen's first new product derived by this recombinant technology is called Raven, and was developed as a superior product for the control of the Colorado potato beetle, as well as caterpillar pests of potato, tomato and eggplant (Carlton, 1996).

Recent investigations aim to obtain transgenic insect-resistant crops by transferring the *Bacillus thuringiensis* toxin gene into plant cells; some successful results have been achieved with varieties of rice, cotton and potato (Xie *et al.,* 1996; CSIRO, 1996; Strauss, 1996).

Besides Bt toxins, other micro-organisms have been studied for future potential as bioinsecticides. More than 100 fungi are known to have entomopathogenic activity and, as biopesticides, they can play a part in integrated pest management. For many years, entomopathogenic fungi such as *Metarhizium, Beauvaria hirsutella* or *Verticillium* have been used for biological pest control with varying degrees of success (Crueger, 1996).

Current research trends are focusing on the development of genetically altered transgenic plants resistant to the action of virus, bacteria and insects. The first generation of transgenic crops will be protected from insect feeding by single genes that encode an insecticidal protein, *i.e.* Bt toxins. Other examples include lectins, protease inhibitors, antibodies, wasp and spider toxins, and insect peptide hormones (Huesing, 1996).

III. DESERTIFICATION

As mentioned above, desertified land provides little support for living organisms. In the case of plants, stress can be defined as any factor that inhibits plant growth. Stress due to drought, waterlogging, salinity, low mineral nutrients, extremes of temperature and pH, and metals, including heavy metals and others such as aluminium and manganese, are common throughout the world. Yet salt stress and drought stress are the most prevalent (Ashraf, 1994).

Salt tolerant plants

Understanding salt tolerance in crop plants remains an urgent issue in plant molecular biology, with a long-term view to genetic improvement. The adaptation of plants to NaCl involves metabolic reactions (synthesis of organic solutes) and transport phenomena (plasmal ion extrusion) (Serrano and Gaxiola, 1994; Dreesmann *et al.,* 1994). Salt-tolerant plants regulate ion transport to tolerate excessive salinity. These plants use energy to overcome the effects of salt

(glycophytes, most crop plants) or exclude salt from tissues (halophytes) (Campbell, 1994).

Using crops, considerable improvements in salt tolerance of important crop species have been achieved in the past two decades using barley, rice, pearl millet, maize, sorghum, alfalfa and many grass species (Ashraf, 1994). Plant scientists have demonstrated genetic variation for salt tolerance in both alfalfa populations and compatible *R. meliloti* accessions, indicating that it may be possible to select and breed salt-tolerant alfalfa populations as well as salt-tolerant *R. meliloti* and other economically important crop species (Campbell, 1994). Such achievements rely on classical genetic breeding techniques and assessments of phenotypic expression of relevant features. However, these studies are also important for future genetic engineering of crops, by combining molecular genetics with classical genetics.

Genetic engineering may provide a general and rapid method for improving salt tolerance in crop plants. The major challenge is the detection and isolation of the key halotolerance genes for use in genetic manipulation. These genes could be components of the normal adaptation response of either crop or halophytic plants to salt stress, and when transferred to plants, their constitutive overexpression could improve salt tolerance.

Another approach is to use simpler models. Micro-organisms are simpler models of gene responses to changes in osmotic pressure (a component of the deleterious effect of salt in plants). Bacteria have characteristic responses, both physiological and genetic, to osmotic stress. The primary response of bacteria to exposure to a high osmotic environment is the accumulation of certain solutes: K^+, glutamate, trehalose, proline, glycine, betamine, at concentrations that are proportional to the osmolarity of the medium (Csonka, 1989).

Two useful microbial models considered in several studies: the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae,* could provide putative halotolerance genes to be transferred to crop plants. This has lead to the isolation and characterisation of the genes proB-74, which determines the accumulation of proline, and HAL1, a modulator of potassium transport (Serrano and Gaxiola, 1994).

Another approach is to detect and isolate genes involved in salt and water stress tolerance using, for instance, protoplast-derived colonies of haploid *Nicotiana plumbaginifolia* selected for resistance to NaCl, KCl and polyethylene glycol 6000 (PEG). These resistant lines can be regenerated into whole plants. As in the case of bacteria and yeast, resistance can be mapped to specific genes and the phenotype of the resistant protoplasts showed a ten- to fifteen-fold increase in production of proline – similar to the findings in bacterial mutants. The use of protoplasts, combined with a well-defined selection procedure, is appropriate for

obtaining stress-tolerant plants and can provide mutants which will be helpful in elucidating the molecular basis of salt stress (Sumaryati *et al.,* 1992)

Recently, a complementary DNA (cDNA) from wheat, HKT1, was isolated, which encodes a high-affinity K^+ uptake transporter (Schacchtman and Schroeder, 1994). The HKT1 was shown to function as a high-affinity Na^+-K^+ cotransporter. Point mutations in the sixth putative transmembrane domain of HKT1 that increase Na^+ tolerance were isolated with the use of yeast as screening system. Na^+ uptake and Na^+ inhibition of K^+ accumulation indicate a possible role of HKT1 in physiological Na^+ toxicity in plants (Rubio *et al.,* 1995).

Finally, nutrient resorption in desert shrubs provides a model of physiological responses resulting from desertification. Nutrient resorption acts to conserve plant nutrients by withdrawing them from tissues undergoing senescence and sequestering them for future use. Desert shrubs may rely heavily on resorption to conserve specific nutrients (phosphorous, nitrogen) that are often in short supply in arid lands. This may provide a focus for future research in important gene expression linked to nutrient resorption (Killingbeck, 1993).

Drought-tolerant plants

Water availability is one of the main limitations of plant growth. In general, the presence of water has been a first-order selective force applying pressure to confer different degrees of drought resistance. In general, all plants (*i.e.* xerophytes and mesophytes), have specific physiological responses elicited by water deficits.

An understanding of the biochemistry and physiology underlying plant responses to water is very important. Today, new ways are being explored in order to alter the genetics and physiology of plants and other organisms with a view to manipulating water use. For instance, it is known that plant gene expression can be regulated by water loss and that some genes are induced by drought.

In order to study gene responses, several strategies have been followed using defined plant models; for example, beans (*Phaseolus vulgaris*), and the simple plant genetic model *Arabidopsis thaliana*. The focus of these studies includes: isolation of genes whose expression is affected by water deficit and/or osmotic stress; isolation of genes which could be involved in osmotolerance; and the study of extracellular matrix proteins which could be part of relevant signal pathways (Covarrubias *et al.,* 1996).

The partial characterisation of different genes, whose expression is induced by drought (many of them induced by phytohormones), has been reported. Results suggest that some water-deficit-induced genes also participate in the processes involved in plant development, for example, plant growth mechanisms

(cellular elongation) which are highly sensitive to water deficit (Covarrubias *et al.,* 1996).

Genetic improvement for drought tolerance could be the long-term solution to grain yield reduction of sorghum. The inheritance of osmotic adjustment to water stress has been investigated (Basnayake *et al.,* 1995). Identification of DNA markers which are associated with drought tolerance may facilitate future sorghum breeding efforts aimed at improving this trait through marker-assisted selection (Tuinstra *et al.,* 1992).

Drought reduces maize production in developing countries on average by 17 per cent, and in some regions the impact can be worse. Drought stress around flowering time typically delays the emergence of silks (silking) up to several days after pollen shedding (anthesis), reducing seed set and yield. Research at the International Maize and Wheat Improvement Center (CIMMYT) has shown that selection for a shortened anthesis-silking interval (ASI) improves maize yield under drought conditions (CIMMYT, 1995). An investigation of grain yield adaptation of advanced wheat lines to water stress environment was carried out in Queensland, Australia, with successful results (Cooper *et al.,* 1994).

These interesting facts, and others reported in the literature, strengthen the idea that an understanding of the genetics of biological processes (both physiological and developmental) involved in drought response will constitute the basis of plant improvement and its potential role in fighting desertification (Covarrubias *et al.,* 1996).

IV. USE AND CONSERVATION OF WATER IN MEXICO

Mexico suffers from water availability problems due to its geographic location and ecosystem distribution. Consequently, water reserves are scarce in some regions, abundant in others. In the big cities, located in the centre and north of the country, where most of the population and industries are found, water is limited. In contrast, the less populated south-west region, which is also the least industrialised, has greater availability of water (Secretaría de Medio Ambiente, 1995).

Economic development and population growth have increased pollution and over-exploitation of the main water bodies, reducing the amount of available freshwater. In addition, most of the effluents (91.5 per cent) generated by different activities (industrial, agricultural, municipal) are returned to the environment without first undergoing proper treatment (Secretaría de Medio Ambiente, 1995), polluting the main water bodies to different degrees with physical, chemical and/or biological agents (Secretaría de Medio Ambiente, 1995).

Agriculture is the main water consumer; about 65 per cent (61.2 km^3 in 1995) of total extracted water is employed in irrigation. Nearly 20 million hectares are dedicated to agriculture and, as a result, two typical water use problems exist: irrigation inefficiency, which creates great water loss; and water quality degradation, due to contamination with pesticides and fertilizers (Secretaría de Medio Ambiente, 1995).

A typical problem is waste water land farming. Some 70 per cent of municipal effluents are used in agriculture without having undergone previous treatment, irrigating 165 thousand hectares. These practices also cause salinisation: nearly 400 thousand hectares (2 per cent of the total agricultural area) are affected by salinity and/or alkalinity problems (Secretaría de Medio Ambiente, 1995).

Over the last seven years Mexico has carried out important reforms in the area of water policies in order to confront a situation of limited supply and increasing demand for water, (Secretaría de Medio Ambiente, 1995; Secretaría de Desarrollo Social, 1993). Bioprevention has the potential to combat a wide range of Mexican water problems, including agricultural concerns and municipal and industrial water management. Efforts should be made to support such technologies because they are important (both immediately and in the long term) in fighting pollution and promoting water conservation at low cost and without undesirable environmental destruction.

V. CONCLUSIONS

In countries with acute water problems water is becoming a factor not only of the quality of life but of socio-political stability – a trend likely to increase as we move into the next century. As the water supply diminishes in terms of availability and quality, societies will become more protective of this resource.

Clearly, the global problem of water supply is exacerbated by human activity. Economic growth and higher demand for water coupled with its mismanagement, have brought about water pollution, soil deterioration, desertification and other maladies, whose ultimate consequences are not only a reduction of available high quality freshwater, but also notorious public health problems in many regions of the planet. Given this near apocalyptic view of the water scenario, it must be realised that more efforts need to be invested into developing new technologies to combat these problems.

Biotechnology offers some important alternatives to the linked problems of water use and conservation. On the one hand, it offers alternative solutions to the short-term problem of preventing water pollution. On the other, it brings the power of genetic engineering to the construction of salt- and drought-resistant crops and

other plants which help in the restoration of land threatened by desertification; a process which, if not arrested, will dramatically affect the world's supply of food.

A great future lies ahead for the integration of biotechnology and the world's water problems. In dealing with this issue, biotechnology has a unique opportunity to fulfil a major societal requirement.

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BIOREMEDIATION: A CHALLENGE TO EDUCATION AND TRAINING

TABLE OF CONTENTS

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SUMMARY

Bioremediation is currently used for the treatment of sites contaminated with various pollutants, most notably petroleum hydrocarbons. As practiced today, bioremediation is a low-tech form of biotechnology, with applications of bioremediation typically relying on the biodegradative capacity of the indigenous microbial populations by using a variety of physical-chemical treatments to accelerate rates of pollutant degradation by those micro-organisms. The main challenge for scientists and engineers is the need for multidisciplinary training so as to predict accurately the rates at which bioremediation will occur at specific contaminated sites, the extent of biodegradation of specific compounds at those sites, and any side reactions that may occur – especially with regard to ecotoxicology and risks to human health. For regulators there is a critical need to understand the capabilities of bioremediation and its complexities so as to determine whether its use at a given polluted site is appropriate. The need for site-specific determinations means that scientists, engineers and regulatory authorities require a high degree of education about this technology, including its strengths and limitations, in order to reach correct decisions as to its applicability. Future development of bioremediation for the treatment of sites contaminated with a broader array of pollutants may require the use of bioaugmentation – that is, seeding with specific non-indigenous micro-organisms, possibly including genetically modified micro-organisms. A thorough risk-based dialogue between the public and the science-engineering communities will be necessary before such deliberate releases of genetically modified micro-organisms could gain regulatory acceptance for bioremediation. Such a dialogue will require greatly enhanced public scientific literacy – which is a major educational challenge to future development of bioremediation and greater market penetration.

I. INTRODUCTION

Although bioremediation has emerged during the past decade as an important technology for the clean-up of some environmental pollutants, it faces many challenges if it is to progress and achieve broader applications. For a general

discussion of bioremediation including descriptions of various applications see the review by Atlas (1995) and the report by the OECD (1994). The successes of bioremediation to date lie mainly in its uses for the clean-up of hydrocarbons and other readily degradable pollutants. The technology typically consists of modifying the environment, by such treatments as aeration and fertilizer application, to optimise conditions so as to achieve maximal rates of pollutant biodegradation. It generally is a low cost alternative to far more costly clean-up procedures.

As currently practiced at the full industrial scale, bioremediation is employed for the restoration of soils and waters contaminated with readily degraded compounds, such as petroleum hydrocarbons, and air contaminated with volatile noxious sulphur- and nitrogen-containing compounds. The most extensive uses of bioremediation have been in the treatment of spillages of petroleum hydrocarbons, such as from marine oil spills and leaking underground storage tanks (Atlas and Bartha, 1992; Bragg *et al.,* 1994; Prince, 1993; Pritchard and Costa, 1991; Swannell *et al.,* 1996). Bioremediation treatments that have been utilised successfully for the removal of petroleum hydrocarbons rely on the natural biodegradative activities of indigenous micro-organisms.

Besides petroleum hydrocarbons, other compounds, including odorous air pollutants associated with animal wastes, are routinely subjected to bioremediation using bioreactors with appropriate microbial populations. Biological treatments are also included in various waste stream treatments for the removal of specific toxic compounds including benzene, toluene, ethylbenzene and xylene (BTEX), and nitroaromatic compounds such as trinitrotoluene (TNT) among others. In many of these bioremediation treatments no seed organisms are applied. Pilot demonstrations have been carried out which have shown that indigenous micro-organisms can be stimulated to degrade trichloroethylene (TCE) and polychlorinated biphenyls (PCBs); besides studies involving micro-organisms and their biodegradative capabilities, plants have also been shown in demonstration projects to be capable of removing heavy metals from contaminated soils and waters (Atlas, 1995; OECD, 1994).

While successful bioremediation can in many cases be achieved without the addition of any exogenous micro-organisms, there are, hundreds of companies that sell micro-organisms as seed cultures for bioremediation. Companies frequently purchase these bioremediation agents. The micro-organisms sold as seed cultures typically have been enriched from natural soils and waters, often at polluted sites so that the micro-organisms in the seed cultures are adapted to the biodegradation of the target pollutants due to prior exposure. Often the micro-organisms in the seed cultures are marketed as mixtures of undefined microbial populations. These seed cultures are essentially sludges containing high numbers of active micro-organisms employed in activated sludge sewage treatments.

They contain micro-organisms that would naturally grow to high numbers given time to adapt to the pollutant chemicals.

The public and regulatory acceptability of bioremediation has rested on its being a natural approach to pollutant removal. It is viewed as a "green" technology that is in harmony with nature. One form of bioremediation, called intrinsic bioremediation, in fact consists entirely of monitoring the natural biodegradation of a pollutant with no treatment whatsoever. In other cases the rates of natural degradative microbial activities are stimulated, often by creating a more optimal environment through simple treatments such as tilling of soil and fertilizer application. As long as the pollutants are quite similar to naturally occurring substances, as are for example the hydrocarbons found in petroleum, this reliance on natural activities works quite well. In many ways bioremediation is an extension of widely used and accepted biological methods applied for sewage and municipal waste treatment. Instead of disposing of human and other animal or plant wastes, however, bioremediation is primarily aimed at the decontamination of sites contaminated with industrial pollutants.

While indigenous micro-organisms can be relied on to degrade many pollutants and bioremediation can be employed to accelerate the rates of biodegradation of those pollutants, there are instances where it would be very advantageous to add exogenous micro-organisms. This form of bioremediation, called bioaugmentation, is critical for those pollutants that are not subject to biodegradation by the naturally occurring micro-organisms at a given site. Some applications of bioaugmentation for bioremediation involve seeding with exogenous micro-organisms that naturally occur at a different site. Other potential applications involve seeding with genetically modified micro-organisms and it is this latter prospect that raises great fears among environmentalists and those regulatory bodies charged with environmental protection. The potential for the deliberate release of micro-organisms, especially if those micro-organisms are genetically modified, requires a great deal of scientific data and public dialogue to perform risk analyses and ensure the safety of such releases.

The reason for considering bioaugmentation and potential applications of genetically modified micro-organisms is that various xenobiotic chemicals (chemicals made by humans through synthetic chemical reactions) evade natural biodegradative processes. The organic chemists have outpaced natural microbial evolution and the choice is to wait for microbial evolution to catch up or to hasten the process of microbial evolution through genetic modification. In some cases these compounds are subject to partial microbial attack leaving residues of incompletely degraded substances. In other cases they are recalcitrant and totally resistant to biodegradation.

Some of these compounds, such as dioxins, are quite toxic and pose immediate threats to human health and environmental quality. It is these resistant and

toxic compounds that are the special targets of research into genetically engineered micro-organisms that might be employed for treatment of contaminated sites. The spectre of deliberately releasing genetically modified micro-organisms, however, has sparked numerous debates relative to the safety of such organisms and their fate in the environment. Both the current applications of bioremediations that employ indigenous micro-organisms and potential future applications that might include the use of genetically modified micro-organisms present challenges with regard to education and training.

II. TRAINING NEEDS FOR SCIENTISTS, ENGINEERS, AND ENVIRONMENTAL MANAGERS

As the scope of environmental clean-up activities increases and the scope of bioremediation broadens in terms of the pollutant compounds and contaminated sites that are subjected to microbial biodegradation for restoration of environmental quality, there is a need to train scientists and engineers who have the necessary expertise to contribute to this effort. This represents an important challenge in an era of increased educational specialisation. Most educational programmes aim at producing scientists and engineers with very high degrees of specialisation rather than the multidisciplinary knowledge required for carrying out effective bioremediation.

Currently multidisciplinary teams of scientists, engineers and managers must work together to provide the necessary expertise. Often, however, there are severe communication problems among individuals who have been trained in vastly different specialities. Each speciality has its own jargon which sometimes confounds communication with other disciplines. Microbiologists, particularly those restricted to laboratory molecular biological manipulations, have much to learn about the environment and its influence on microbial activities. Engineers, who are often responsible for environmental clean-up activities and who need to develop models for predicting the fate of pollutants subjected to bioremediation treatments, have much to learn about micro-organisms. Scientists and managers charged with the oversight of environmental clean-up need to incorporate risk analysis that takes into account the hazards posed by environmental pollutants, micro-organisms that may be employed for bioremediation, and residual products that may remain after such biological treatments. These activities require a breadth of knowledge of multiple fields including molecular biology, hydrology, statistics, mathematical modelling – all pointing to the importance of developing a new approach to multidisciplinary training related to bioremediation.

It is unlikely that the current higher educational system that produces research scientists, engineers and managers will provide the necessary training

programmes as part of the traditional system that is discipline-based. University administrators who have experimented with multidisciplinary programmes often point to difficulties in placing students in appropriate employment positions. Many industries that profess a need for multidisciplinary scientists continue to hire specialists with the best records of academic performance and other experience, preferring to subsequently offer additional training to improve job performance. Thus the challenge will be to develop and implement postgraduate and postemployment courses that will provide the necessary training for developing expertise in bioremediation.

Several component modules are critical for inclusion in the training of scientists, engineers and managers involved in environmental clean-up activities that employ bioremediation. These components include:

- *Environmental sciences:* Training in hydrology and other areas related to environmental transfers and transport of micro-organisms and environmental contaminants is important for understanding the movement of chemicals in soils and waters.
- *Chemistry:* Training in analytical methods involved in detecting and determining the concentrations of pollutants and the interpretation of various analyses is critical for environmental monitoring and understanding the environmental fate of pollutants.
- *Microbiology:* Knowledge of microbial ecology and physiology is necessary for understanding the capacities of micro-organisms to degrade pollutants, including limitations to natural biodegradative activities.
- *Toxicology:* Training in human toxicology and ecotoxicology is important for understanding the risks associated with pollutants and any by-products of biodegradation.
- *Molecular biology:* Training in molecular biology is important for the production of micro-organisms with novel physiological characteristics, including metabolic capabilities that may be useful for bioremediation of persistent and toxic xenobiotic chemicals.
- *Statistics:* Training in the methods involved in experimental design and interpretation of analytical results is essential for determining the efficacy and safety of bioremediation.
- *Modelling:* Training in mathematical models is necessary for predicting the environmental fate of chemicals and predicting the effectiveness of bioremediation.
- *Environmental law:* Training in environmental law and regulations is necessary for determining the requirements of environmental clean-up procedures and the applicability of bioremediation.
- *Economics:* Training in economics is important for analysing the costs and benefits of bioremediation relative to alternative remediation technologies.

- *Risk analysis:* Training in risk analysis is critical as regulations move from absolute clean-up levels to risk-based clean-up performance criteria.
- *Communication:* Training in communication is essential for conveying information to scientists from different disciplines, engineers, managers, regulators, policy makers and the public.

In addition to the need for alternative curricula to provide the necessary breadth of expertise, there is a need for training at the international level, including the multidisciplinary training of scientists, engineers and environmental managers in developing countries. This is especially important as developing countries attempt to solve pollution problems and to develop biotechnology industries based upon indigenous biological diversity. The necessary decision making about the applicability of bioremediation requires such knowledgeable individuals within the nation and locality where the clean-up activity must occur. In many nations this will require capacity building to establish the necessary trained personnel needed for the infrastructure involved in regulatory and environmental managerial decision making.

III. CURRENT AND FUTURE EDUCATIONAL NEEDS

When bioremediation is proposed as a method for the treatment of contaminated sites, there is a need for understanding the underpinning microbiological and environmental sciences, both at the public and scientific/engineering levels. In most cases this requires enhanced education regarding the strengths and limitations of bioremediation, especially when the treatment involves the introduction of exogenous micro-organisms. The public is largely unaware of the benefits of micro-organisms – having focused far more on the detrimental aspects of micro-organisms as human and plant pathogens. The public often fears any exposure to micro-organisms and considers all micro-organisms to be causes of disease, whereas in reality many micro-organisms carry out beneficial activities upon which all life depends. Changing the popular view of micro-organisms through educational programmes that increase scientific literacy, is critical to the public acceptance of bioremediation.

Public education is essential because, in the end, bioremediation requires public acceptance. Increasing public literacy about science and specifically biotechnology is especially important as risk analyses become pivotal in the decision-making process about the extent of clean-up required and the technologies that are selected to achieve those clean-up levels.

A fundamental aspect of the scientific literacy that must be achieved is the methodology used by all scientists or hypothesis testing based upon observations

and controlled experiments. It is important that the public as well as environmental managers understand that scientists cannot provide absolute answers. This is because the scientific method, the fundamental philosophical basis for all scientific investigations, is based upon hypothesis testing which rejects incorrect hypotheses rather than proving correct ones. Thus scientists can demonstrate things that are not true and by doing so gain a high degree of confidence in the correctness of other explanations, but they can not determine the absolute correctness of those hypotheses which are not rejected – there may always be alternative correct hypotheses that have yet to be tested and hence always some degree of uncertainty. The potential for alternative hypotheses that cannot be rejected is why scientists are unable to make categorical conclusions in absolute terms. One can always raise conjectural alternatives that, however unlikely, would have to be tested.

Debates about the safety of biotechnology are often based upon conjectural-philosophical arguments that have no scientific validity. Claims that scientists who carry out genetic engineering will inadvertently create an "Andromeda strain" that will destroy all human life or devastate the world's biodiversity are too frequently put forward by environmentalists who oppose modern biotechnology. Equally unsupportable claims are made by various entrepreneurs about the benefits of biotechnology. Some companies claim to have a micro-organism that will completely degrade any environmental pollutant within a few hours. Too often such claims about the capabilities of bioremediation are made without conducting the necessary scientific experiments to substantiate those claims; many of the claims about the successful applications of bioremediation agents and technologies are based upon laboratory and/or field demonstrations that lack controls.

The ability to critically evaluate the scientific merit of claims about the safety and performance capabilities of bioremediation technologies is necessary for effective managerial decision making and environmental management practices by those charged with ensuring the effective and safe removal of environmental pollutants. Regulators and environmental managers must understand the fundamental scientific basis of bioremediation in order to reach sound judgements about the uses of this technology.

Beyond the need for increasing the general level of public scientific literacy and the more specific knowledge about bioremediation among environmental managers and those charged with the implementation of environmental regulations, there is a continuing need to enhance the knowledge base of policy makers about environmental biotechnology. New educational paradigms are necessary to get scientific knowledge to policy makers in useful and understandable forms. As the multidisciplinary fields of science involved in environmental biotechnology become more precise, there is a tendency to use terms whose meanings are not readily understood by policy makers. There is a particular problem with the use of

jargon that is understood by only a limited group of scientists. This leads to an isolation of scientific knowledge and a lack of communicability. The result is the development of public policy that is not based on adequate and integrated data.

Attempts to translate the scientific information about environmental biotechnology that is contained in reports and peer-reviewed literature into forms that can be understood by policy makers often result in oversimplification and confusion. This communication problem highlights the need for communication training for scientists, engineers and environmental managers. In addition to learning how to communicate with the public and policy makers, scientists must increasingly take the time to establish dialogues with the non-scientific community. While this will detract from the time available to develop new knowledge in the laboratory or in field experiments, it will greatly aid in the advancement of environmental biotechnology and help ensure that appropriate research directions are followed and that there will be adequate funding for future research. Dialogue with the public and environmental policy makers will aid scientists to focus research on areas of concern and ensure that research is conducted in areas that will help define a policy that ensures the appropriate roles for environmental biotechnologies in environmental protection and remediation of contaminated sites.

In order to establish appropriate environmental policies and to make correct decisions about the applicability of bioremediation, it is important not only to have an educational background that ensures scientific literacy and the ability to make informed management decisions, it is critical to have access to the latest information. This makes informational resources an essential component of all continuing education systems. This will remain especially critical as long as bioremediation remains based on case-by-case demonstrations. Avenues of information currently available include the open scientific literature and numerous scientific conferences, some of which are exclusively devoted to bioremediation.

Perhaps more important are databases that can be accessed through the World Wide Web. Several databases are available to aid in the formulation of environmental policy and the enactment of managerial decisions relating to potential applications of bioremediation. These include databases describing in some detail the applications of bioremediation that have been performed and scientific analyses of field demonstrations. These case descriptions indicate the scope of applicability of bioremediation and serve to guide environmental managers in decision making. Additionally there are databases of environmental regulations which are important for providing information needed by environmental managers. Information in these databases also serves as an aid for national formulations of policy regarding the applicability of bioremediation and decisions about investment in research and development in environmental biotechnology.

Knowledge of environmental regulations, including those relating to the import, export and deliberate environmental release of micro-organisms, is impor-

tant for international trade of bioremediation agents and technologies.The shipment of such micro-organisms must comply with internationally accepted standards and their import, export and utilisation must meet national requirements which typically involve a permit procedure. Currently many microbial cultures or mixtures of micro-organisms can be used for bioremediation on a commercial scale as long as they are shown to be free of plant and animal pathogens and are not genetically modified. As the scope of bioremediation increases and genetically modified micro-organisms become available which could be considered for deliberate release into the environment, access to current international regulations will be even more critical for those responsible for policy formulation, for investors in environmental biotechnologies, and for the environmental managers who may decide to use modern biotechnologies for bioremediation.

Complicating decisions surrounding the applicability of bioremediation for the clean-up of a given pollution problem is the fact that the scientific community has yet to reach a point where generalised principles can be applied. One of the problems with developing a general prediction capability for bioremediation is that many of the detailed scientific studies concern the biodegradation of individual compounds, such as trichloroethylene, or individual classes of compounds, such as polychlorinated biphenyls; however pollutants generally occur as mixtures and in real-world contaminated sites there are numerous interactions that determine the fate of those polluting chemicals. Scientists have yet to develop an understanding of these interactions in ways that yield reliable predictability about bioremediation that can be extrapolated from one site to another.

The practice of bioremediation, therefore, remains something of an art. More information, particularly field performance data, is needed to increase the predictability of the performance of bioremediation, especially at the full-scale engineering level. Problems with scale-up are often cited as a severe limitation to bioremediation. Increased knowledge, some of which must be gained by further research and development, is currently necessary. Additionally the factors influencing the effectiveness of bioremediation are highly site-specific. Bioremediation for the treatment of many pollutants remains dependent upon field demonstrations that allow the evaluation of how well this technology will perform, as well as providing the necessary data for effective scale-up and prediction of performance at a given environmental site.

A second area of complication that presents special educational challenges to policy makers, regulators and environmental managers is that bioremediation is not an instantaneous process and is often incomplete. Risk analyses must take into account the time a pollutant will remain in the environment and what will remain after using bioremediation as a clean-up technology. This involves analyses and, in the end, value judgements about land uses and acceptable risks.

Reaching appropriate decisions requires information and critical evaluations by an educated public and decision makers through informed dialogue.

IV. CONCLUDING REMARKS

Although bioremediation has already become a routine practice for the treatment of some pollutants and some contaminated sites, challenges remain – especially in the areas of education and training. As attempts are made to expand the scope of bioremediation to sites contaminated with a greater variety of pollutants, there will be increasing challenges to education and training of those involved in environmental remediation. In particular there is a need for new paradigms in training scientists, engineers and environmental mangers in the multidisciplinary aspects of bioremediation which differ significantly from the trend to train specialists. Information communication across conventional disciplinary lines is critical – including communication with the public about the benefits and limitations of bioremediation. There is also a critical need for enhanced public education to increase scientific literacy. This is essential for the necessary dialogue between the public and those charged with formulating environmental policy and overseeing the clean-up of contaminated sites. Risk analyses aimed at determining acceptable approaches to environmental remediation depend upon informed, value-based discussions that consider costs and benefits, as well as technological capabilities and limitations. Any breakthrough developments of bioremediation based on genetically modified micro-organisms will require a high degree of enhanced scientific literacy and careful risk analyses for commercial developments that could improve environmental quality.

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