Biotechnology: Opportunities and Constraints

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This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

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Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.
BIOTECHNOLOGY: OPPORTUNITIES AND CONSTRAINTS

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FOREWORD

The following text was written for the Board of Governors of the International Development Research Centre. It presents some important issues relevant to what is broadly described as "Biotechnology". It raises issues of concern to IDRC in its relations with developing countries many of whom have been presented with extensive, in some instances unduly optimistic accounts of what "Biotechnology" has to offer.

This document is less about research than about the resources needed if research in certain of the biosciences is to yield benefit to developing nations or to anyone else for that matter. It seeks to bring into focus the downstream issues and the complex assembly of physical, material, financial and human resources that will be needed if bioscience research is to metamorphose into biotechnologies: technologies which combine practical utility with economic and social acceptability among clearly defined populations. These are the issues to which IDRC proposes to give greatest attention in the near term.

Joseph H. Hulse
Vice-President Research Programs
International Development Research Centre
# BIOTECHNOLOGY

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*Contents prepared by IDRC staff unless otherwise indicated.

**This paper contains portions of a report prepared for the Science Council of Canada.
BIOTECHNOLOGY

INTRODUCTION, OVERVIEW AND POLICY ISSUES

INTRODUCTION AND OVERVIEW

"Biotechnology" now embraces so extensive, expansive and diverse a spectrum of biological principals, phenomena, materials, organisms, reactions and transformations that no longer can it logically be considered in the singular as a collective or mass noun. To add further complication, much that is so named appears some distance from applicable technology as the term is generally understood. This comment is intended not as a semantical quibble but to caution against the concept that biotechnology comprehends a concise and discrete communion of activities sufficiently homogeneous and inter-related to be conveniently assembled within a single institutional research and development framework.

This brief commentary, together with its several appendices will attempt to illustrate the heterogeneity of the subject and the wide diversity of products and services its many components may offer. It will seek the Board's guidance upon certain policy issues relative to the Centre's present and future program activities and to the growing number of requests for support and advice coming from developing countries. While it is issues of policy the Board is invited to address, the relevant scientific principles and technical considerations cannot be ignored. In order to minimize the hazards of technical indigestibility, most of the technical content is contained in the appendices which follows this discussion paper.

Since the single common thread running across the biotechnological spectrum is biology: the nature, behaviour, components, composition, conversion, mutation, transformation and utilization of living cells, their structural and constituent materials, the last two appendices consist of a brief description of the structure of living organisms and a glossary of terms that will be encountered in some of the appendices and other related literature.

Other appendices describe the products and essential resources of various fermentation industries; research, development and manufacture of certain vaccines and hormones (written by a senior scientist in a prominent Canadian company); cell and tissue culture in plant breeding; some legal considerations; information sources; a bibliography of some documents collected in the Centre; a brief review of related activities in Canada and selected developing countries; activities related to biotechnologies supported by IDRC.

The contents have been compiled from a very large volume of literature, from many interviews with research workers, industrial scientists and technologists, with senior policy makers and planners in several developing countries and a variety of other sources, public and private. These presentations make no claim to be comprehensive. The related literature is enormous and growing rapidly, and to seek it out requires reference to a variety of data bases.

....2
Few if any scientific subjects have so caught the attention of the news media. Given the extreme esotericity and scientific complexity of biological research it is perhaps not surprising that journalistic attempts to popularize the subject have tended as much to delude as to enlighten. Thus among the massive literature it is oftentimes difficult to separate fact from fiction: to differentiate between wishful thinking and informed, reliable, rational and responsible judgement. More is written about laboratory research than about confirmed, economically sound technological applications. In many instances, therefore, "biotechnology" is a misleading descriptive since the technologies discussed are sometimes more speculative than real.

Responsible and active research workers and people directly engaged in biotechnological industries present a more cautious attitude towards what may be accomplished, the time-scale to reach practically applicable objectives, and the scale of resources needed. As one of them stated "this is not a field for two engineers in a garage".

Certainly many of the benefits to be realized from deliberate genetic transformations will take longer to deliver and require greater investments of money and skills than originally forecast. Professor Ausubel of the Harvard Medical School, who first reported the successful cloning of a single cluster of nitrogen fixing (nif) genes, predicts it will take between 10 and 50 years to generate a nitrogen-fixing maize plant. More of the published literature is devoted to research and laboratory techniques than to the organizational structures and downstream resources needed to distribute and deliver the products of research to intended users in a safe, effective and economically sound fashion. This is evidenced in several developing countries which propose national institutes of biotechnology in advance of any comprehensively conceived plan of downstream development and implementation.

Industrial fermentations offer an immense range of potential products, products to serve widely diverse markets and end-users. Some are described in Appendices I and II. The successful enterprises are those who have specialized in carefully selected markets. The experience of major North American biological research institutes has indicated the constraints experienced when applied research is inadequately integrated with market research, marketing, distribution and manufacturing facilities. There is a clear concensus among those interviewed who serve the industrial sector that a well-defined demand pull provides a more reliable guide to success than a laboratory inspired technological push. The author of the paper on vaccines and hormones (Appendix II) convincingly argues that research to develop such products can easily go off-track in the absence of constant feedback from the factory floor, from the marketplace, from clinical trials and from monitored experience with patients.

Similarly applied research on plant cell and tissue culture will likely be most productive when integrated into existing national plant breeding and crop improvement programs (Appendix III). Tissue culture will continue to complement and expand the techniques and resources available to plant breeders. To achieve this goal the research needs to be guided by and intimately integrated with established plant breeding programs...
Both Appendices I and II indicate that research represents but a modest component of the total package of resources needed to bring any product of biotechnology to a receptive market. Pharmaceuticals and other health products are particularly demanding of long-term investments in financial, material and skilled human resources. As stated in the appendices, skilled and experienced human resources are particularly scarce, the demand for biological engineers being greater than the supply wherever biotechnological industries exist or are being developed. Most critical is the shortage of competent experienced managers of research, of production and marketing, and senior executives capable of planning and directing the complex systems that make up any biotechnology industry.

Few if any nations possess the resources to pursue all that applied biological science might conceivably offer in the future. The large established pharmaceutical companies will probably concentrate upon high value drugs which can be afforded by aging wealthy customers, in addition to expanding the existing markets for many of the biologicals referred to in Appendix I. The chemical companies see fermented biological materials as economic alternatives to petrochemical feedstocks. Seed companies and departments of agriculture in North America, Europe and Oceania will use tissue culture to accelerate and refine the development of improved crop types. Veterinarians will gain access to a larger arsenal of diagnostic, prophylactic and therapeutic agents.

To the question "What has biotechnology to offer the developing countries?" there is no simple answer. The more advanced already possess pharmaceutical and chemical industries upon which to build, expand and diversify. Many, however, are having difficulty in arriving at a cogent, coherent national policy or plan for biotechnology. The popular literature has suggested to them that biotechnology research will bring rapid improvements in human and animal health; provide cheaper energy resources; higher yielding plants, more resistant to drought, pests and diseases. One Asian national newspaper proclaimed "We now have a technology which can satisfy all man's needs". It is evident that a number of developing countries to whom biotechnology has been presented as a panacea for many of their ills need helpful guidance if those responsible for science and technology policy are not be misled into scientifically intriguing but economically unrewarding research investments.

POLICY ISSUES

The Centre's Role: Active or Passive

Biosciences are fundamental to many of the projects IDRC has supported. As new proposals are presented it will be for the Divisional Directors responsible to determine their respective merits and to recommend whether or not they should be approved and accepted. However, while remaining in general responsive to the requests submitted by developing countries, a greater or lesser involvement in any sector can be encouraged or discouraged. The Centre can be active, seeking a leading role as it has in selected areas in the past, or it can remain passive, awaiting but not encouraging project proposals.
Applied or Basic Research

Early in its history the Board recommended that the Centre concentrate its support upon applied research: research designed to bring eventual benefit to a defined community of recipients. Does the Board wish to reaffirm this recommendation?

The question is germane since a considerable proportion of what is labelled "biotechnology" has been arrived at empirically. To achieve significant new progress in many sectors requires more fundamental studies. Should the Centre devote any of its resources to longer term basic research either in Canada or in developing countries with a demonstrable competence? Or, should support of such research be left to other agencies?

The issue of institutional and program support has often been raised in the past. So far, the Centre has not clearly defined what is intended by institutional and program support, what are to be its scope and limits. Several countries have requested support to build their national biotechnology programs; for training in laboratory and pilot plant techniques; for what those making the request regard as essential equipment.

Sectoral Choices

If the Board reaffirms that the Centre's resources remain strictly dedicated to applied research, it should not be difficult for the Centre to make appropriate choices in the crop improvement sector. Appendix VII reviews various types of information service the Centre could provide if so required and provided with the necessary resources. Several activities in the Health Sciences program are of direct relevance including the diagnosis and control of tropical and infectious diseases, particularly the vaccine-related studies; health operations research - the difficulties and constraints in the downstream health delivery systems are likely to prove even more intransigent than the laboratory development of new drugs; water and sanitation. In the latter case it must be remembered that all fermentation industries generate their own waste products many dissolved or dispersed in water which is often allowed to pollute public waterways. Furthermore containment and human safety are of continuing concern particularly where genetically transformed pathogens are involved.

Industrial Development

The Centre lacks experienced expertise in the industrial fermentation sector. It possesses relatively little in-house practical experience in biological engineering, in either market research or the marketing of manufactured products. It is in these skills that many developing countries are also deficient. Yet it would appear fruitless to pursue research to develop new
products of fermentation before first determining what potential markets exist and what resources are needed to manufacture and deliver them to the markets defined.

As indicated in Appendix I, the range of products from fermentation industries is very great indeed as is the variety of marketing channels through which they are distributed. Each market presents its own challenges and requires specialized knowledge and experience. At present it is beyond IDRC's resources to give advice in market research or marketing in any branch of industrial fermentation. If however the Centre is encouraged to give greater support to industrial development it must consider seriously how to provide the essential elements of market research.

The Centre has given modest support to the improvement of traditional food fermentations. This may be a sector for possible expansion. It has several times been suggested that attention be given to the more efficient use of abattoir by-products which, in North America, are a prime source of fine chemicals. Some developing countries might find the adaptation of existing processing technologies for animal by-products a more convenient route to fine chemicals manufacture than fermentations with genetically transformed microorganisms.

It is obviously beyond the Centre's present resources to engage directly in the establishment of new biological industries in developing countries. The Centre has however established professional contacts with many scientists and technologists who possess extensive experience in industrial fermentation industries. It has therefore been suggested that IDRC sponsor one or two regional workshops at which selected consultants could advise those responsible for science, technology and industrial planning of the resources and facilities needed to establish economically sound fermentation industries. If the Centre agrees to this course of action, it has firm offers of cooperation from several professionals experienced in the development, manufacture and distribution of human and animal vaccines, fine chemicals and organic chemicals.

Human Resource Requirements

Centre staff have frequently written and spoken about the importance of resource allocation studies. In any branch of science and technology it is the skilled human resource which is most critical. As developing countries engage in new ventures and enterprises their demand for skilled people increases. All new activities in bioscience and biotechnology become competitors with existing sectors for an inadequate supply of people trained in the natural and physical sciences.

The time may have arrived for the Centre to devote its attention, perhaps using regional office funds and the resources of the Science and Technology Policy program, to assist government planning agencies systematically to assess the present and future supply and demand for skills essential in those sectors of science and technology to which developing countries are giving priority. Without suggesting the Centre assume a principal role as an advisor to governments on bioscience and biotechnology policy and planning, a sizeable body
of knowledge and experience exists within IDRC which, without prejudice to the Centre's main activities, could be made available to developing countries through regional workshops and seminars. The two papers on legal considerations (Appendices VI and VII) add a valuable dimension to the technical considerations and demonstrate the breadth of the Centre's relevant competence.

The More and Less Developed

Several nations, largely in Asia and Latin America, are continually progressing in their development of industrial infrastructures. Investment in applied research promises hope of further progress and economic benefit among these front runners. But what of the lesser developed nations, those with only primitive industries, health delivery systems inadequate to make safe and effective use of new drugs, let alone contemplate their development and manufacture; too few trained people to staff the most basic essential technical services. For some of the poorest, bioscientific research and development will have to wait until more immediate difficulties are overcome.

There are nations which lie somewhere in between. Which with adequate resources devoted to training, to institutional and infrastructural development might begin to derive benefit from bioscientific research and development. Many of these do not know quite where to begin; they are struggling to develop a science and technology policy framework. They urgently need advice upon what human skills and other resources are necessary to develop and benefit from bioscience and biotechnology.

Should IDRC concentrate its limited support upon the most advanced; those who can conduct applied research and exploit its results? Or should the Centre have concern for the scientifically less developed and help them over the first hurdle towards developing a relevant and realistic science policy, and to an assessment of the resources they possess and the resources they will need?

Finally, since Canadians are presently struggling to define a national biotechnology policy, the Cooperative Program might examine in what manner Canada's emerging philosophy and experience could usefully be made available to the developing world.
APPENDIX I: Industrial Fermentation

A brief review of various classes of industrial fermentations: pharmaceuticals, industrial chemicals, food, agriculture and other applications. Opportunities, constraints and essential resources are discussed.

APPENDIX II: Research, Development and Manufacture of Vaccines and Hormones

A comprehensive account of the institutional, physical, material, human and financial resources and the time scales required in the development, testing, distribution and application of vaccines, hormones and related human health products. The contents are based upon the contemporary experience of a prominent Canadian organization with integrated facilities for research, development, manufacture, animal and clinical testing, distribution, quality control and monitoring. Reference is also made to safety standards and conditions of containment.

APPENDIX III: Plant Breeding, Cell and Tissue Culture

A brief review of conventional plant breeding methods and the various alternative techniques of cell and tissue culture that have been or may be useful in plant breeding. The different stages of progress among different techniques are briefly reviewed. The transfer of excised DNA between plants is much more difficult than the genetic transformation of bacteria. Much of the progress described has been reached empirically and a more complete understanding and control awaits fundamental research of an exceptional degree of imagination, skill and competence.

APPENDIX IV: Biotechnology: New Horns for an Old Dilemma

A historical review of the development of various industrial biotechnologies; some recent advances and difficulties encountered in establishing economically sound biotechnological development; the need for a new generation of managers of science and technology.

APPENDIX V: Biotechnologies: Responsibilities, Priorities and Constraints

A review of priorities and constraints in the research and development of biotechnological products in developing countries. Consideration is given to the resources needed to realize useful and deliverable biotechnological products from the myriad of research subjects now being proposed. The need for planning research priorities is discussed in view of resource constraints.
APPENDIX VI: A Regulatory Framework Governing Biotechnology

The leader of an IDRC supported project discusses the mechanisms by which the processes and products of biotechnology may be regulated. The discussion addresses why mandatory and/or self-imposed regulations are necessary; what aspects may need to be regulated; by whom regulatory standards and protocols may be formulated and particular implications for Third World countries.

APPENDIX VII: Legal Considerations in Canada

A review of relevant legal processes and responsible agencies in Canada.

APPENDIX VIII: Information on Biotechnology

A commentary upon the diversity of information sources and data bases relative to the subject together with some possible courses of action for IDRC.

APPENDIX IX: IDRC Bibliography

A list of relevant publications collected by IDRC up to the end of 1984.

APPENDIX X: Biotechnology Activities Funded by IDRC

An illustrative account of some projects and activities related to various facets of biotechnology that have been supported by the Centre.

APPENDIX XI: The Structure and Reproduction of Living Organisms

A brief review of the structure, composition and behaviour of living cells: the nature of genes and the genetic code and the processes by which DNA reproduces itself and by which genes can be isolated and pieces of DNA transferred between organisms.

APPENDIX XII: Glossary

An attempt to provide definitions and descriptions of many of the esoteric and scientific terms used in the biotechnology literature.
SCOPE AND DIVERSITY

Until WWII the organic chemical industry was built on coal, coal tar derivatives being the starting materials for dye stuffs, synthetic resins, together with many other chemicals used in pharmaceuticals, cosmetics, and food industries. Following WWII, petroleum by-products largely replaced coal. At the start of WWII sixty percent of organic chemicals were derived from coal; by 1980 almost 80% had their origins in petroleum feed stocks.

In recent years both chemical and biological agents have been used more extensively in petroleum by-product conversions. As oil costs have risen, industry has looked more intensively towards biomass of cultivated and natural occurrence as a potential feedstock for the manufacture of many chemicals. Since biological conversions generally proceed at relatively lower temperatures than chemical reactions, they offer the attraction of potential fuel savings.

Industrial fermentations employ biological agents to convert biological materials from one form to another. In terms of total tonnage the greatest application is to waste management including water and sewage treatment. The biological agents employed include cultured microorganisms, plant and animal cells and isolated enzymes. In terms of economic value, alcoholic beverages, followed by dairy products, bakers' yeasts and organic acids are the industrial leaders. Among fine chemicals antibiotics (valued at close to $5 B U.S. annually in the United States alone) are the leaders.

Many traditional foods are produced by natural fermentations (see Appendix IV) most having been in human use long before microbiology and biochemistry became systematic sciences. Essentially, industrial biological conversions take advantage of the natural metabolic processes of living cells. Over much of history people utilized the metabolic end products of microbial fermentations as they found them. Yeasts of the Saccharomyces genus convert simple sugars mainly to ethanol and carbon dioxide, the former having been of primary interest to brewers and vintners, the latter to bakers in leavening their bread for over 6000 years.

Manipulation of Microbes

In time it was found that microbial cells could be manipulated to change the nature and concentration of their metabolites. For example during WWI German scientists discovered that by adding bisulphite the metabolic pathway of yeast-sugar fermentations could be changed to produce glycerol rather than ethanol, the former being used in the explosive nitroglycerin (Appendix IV).

By selection among strains of the same species, microbiologists isolated those which produced higher yields of the final or intermediate metabolites of interest to them. By irradiation or chemical treatment mutant strains were produced and selected for desirable superior characters. By strain selection and progressive improvements in the culture media, yields of, for example, antibiotics have been significantly and progressively increased.
By fine tuning of the bacterial strains in the laboratory media in which they are cultured a very wide range of economically useful biologicals is now possible. Given that out of the many thousands of microorganisms identified and classified, relatively few are employed industrially, the future for industrial fermentation seems one of continuous extensive expansion.

Hope for more precise and varied application of microbial fermentation looks to genetic transformations: the transfer and incorporation of exotic fragments of DNA either to increase the production of normal metabolites or to stimulate the biochemical synthesis of substances foreign to the organism in its natural state. For most of man's history fermentations have been carried out in batch systems. In some suitably discrete container the microorganism and its nutrient medium interact until the metabolites generated reach levels of concentration at which the process is arrested. For example the maximum concentration of ethanol which most fermenting yeasts will tolerate is between 10 and 12 percent. The maximum achievable concentration for some antibiotics is below 5% and even lower for several other metabolites such as riboflavin and other vitamins.

Continuous Fermentation

Batch fermentations are generally considered uneconomic in modern industry, so that brewers and other fermentation industries have devised continuous fermentations in which the dissolved substrates to be converted are passed in continuous flow over microorganisms or enzymes held in static immobilization in reactor columns (see Appendix IV). The efficiency of the techniques by which the cells or enzymes are held immobilized, the concentration and rate of flow of the media solution determine the rates of production of the desired end products and the overall efficiency of the process. Significant yield improvements over traditional batch processes are reported for many fermented products. Published reports indicate that butanol and acetone from continuous fermentation with immobilized bacteria give yields 200 times higher than batch fermentations. Continuous flow over immobilized biological catalysts is applicable both to product synthesis and to the purification or detoxification of effluents and aqueous wastes. Technologies which employ immobilized enzymes and microorganisms are relatively new and still evolving (See Appendix IV).

Products of Fermentation

The products of industrial fermentation fall into several broad classes:

(a) Pharmaceuticals and Health Biologicals

(i) Diagnostics including monoclonal antibodies, other immunoproteins, enzymes, DNA probes for detecting genetic abnormalities in foetuses;

(ii) Prophylactics including antibodies, antigens, vaccines;

(iii) Therapeutics including antibiotics, hormones, steroids and enzyme inhibitors.
(b) **Industrial Chemicals**

(i) Bulk chemicals including alcohols, organic acids, aldehydes, ketones and simple molecules such as ethylene, methane and peptides from which more complex polymers can be synthesized;

(ii) Fine chemicals such as hormones, enzymes, steroids, polysaccharides and peptide polymers.

c) **Agriculture**

(i) Traditional processes include ensilaging and composting;

(ii) Manufactured animal feeds generally contain various antibiotics, amino acids and vitamin supplements;

(iii) Steroidal hormones; recently bovine and porcine growth hormones are reported from recombinant DNA techniques;

(iv) Diagnostics, prophylactics and therapeutics similar to those listed above under 'pharmaceuticals' including monoclonal antibodies, specific antigens, veterinary vaccines;

(v) Microbial pesticides: these include pathogenic bacteria and toxic substances specifically applicable to the control of known microbial and insect pests and for weed control;

(vi) Rhizobial and mycorrhizal inoculants to improve the uptake of nitrogen and other plant nutrients.

(In some literature embryo transplants in cattle are sometimes listed under "biotechnology". These are considered no more logically admissible than artificial hearts and joints for humans.)

d) **Food**

(i) Appendix IV describes various classes of traditional fermented foods;

(ii) Industrial fermentations produce an immense range of food additives: nutrients such as amino acids and vitamins; pigments, flavours and flavour enhancers; preservatives; carbohydrate derivatives; modified sugars, synthetic sweeteners; structural and protective colloids, emulsifiers and other texture modifiers;

(iii) Microbial protein from fermented carbohydrates and petroleum by-products and various biological materials.

e) **Other Chemical Applications**

In addition to their use in foods, several of the product types mentioned are used in cosmetics, toiletries and non-prescription medicinals. For example the polysaccharide xanthan, produced by
Xanthomonas species, is used to increase the viscosity of food products such as mayonnaise, to a similar end in cosmetics and paints, and as a replacement for cereal starches in the chemical muds of oil wells. Genetic manipulation of microorganisms promises a greater range of macro-molecules for technical use and as starting materials for various chemical conversions.

Bacteria able to convert sulphur compounds are used in increasing the desired metallic content of mined ores of copper and uranium, and in the processing of effluents from pulp and paper manufacture.

The potential use of immobilized bacteria and enzymes for the purification of wastes, effluents and the removal of toxic and noxious substances is greater than yet realized. Other applications foreseen include the removal of unwanted substances from body fluids and the addition to human blood of enzymes, hormones and other essentials in which they are deficient.

(The fermentation of biomass to generate energy sources will be reviewed in the Energy Research Group's publications and therefore excluded from these discussions.)

Opportunities and Constraints

The future applications of microorganisms and isolated enzymes in industrial biochemistry and organic chemistry appear almost limitless. So extensive is the potential that the most taxing difficulty will be to select wisely from the vast smorgasbord laid before research institutions and science and technology planners.

To address all of the potential opportunities, the necessary resources and constraints attendant upon each of the vast array of bioscientific possibilities would demand more space, time, comprehensive and contemporary hands-on experience than is available within the Centre or to the writer. Furthermore, the opportunities and constraints vary significantly among regions, countries, communities, manufacturing, marketing, research development, service, government and non-government organizations. Therefore very few if any general recommendations can be made to all developing country governments interested in this fascinating field. Indeed one of many hazards lies in the probability that several will pursue similar objectives and product developments only to find that markets are not large enough to accommodate all who would seek to serve them.

All this notwithstanding, following correspondence and conversations with a large number of scientists and organizations directly involved and from a sizeable volume of literature reviewed, some general comments are offered below. First and foremost it cannot be too heavily emphasized that the actual and potential range and applications of industrial fermentations are enormous and cannot be comprehensively addressed in a single review. Each needs to be considered systematically and independently according to the
markets and end-uses its products will serve, the raw materials and other resources to be employed and, most important, the human skills and investments essential to success.

Though the two facets should be complementary, a distinction is necessary between applied biological research intended to produce a commercially viable technology or essential service, and basic research which seeks a deeper understanding of the biochemistry and physiology of living organisms. While the latter may well be pursued successfully in such discrete research laboratories as national institutes of molecular biology, technology development requires intimate integration of research and development with the facilities for market research and identification and with the skills and resources essential for gradual scale-up from the laboratory in vitro to the in macro of the manufacturing unit.

Though a considerable investment in basic biochemistry and plant physiology research will be needed before much of what is discussed can progress from the purely empirical to the systematically scientific, the organization and the pursuits of biological research laboratories engaged in basic research will not be addressed in this presentation.

Appendix II presents an account of the complexities of developing and manufacturing vaccines and hormones for human use from genetically manipulated microorganisms. The literature which popularized and presented biotechnology as a probable panacea for many human and industrial ills gave little regard to the difficult technical problems, the resources of cost, time and human skills required to progress from a single genetically altered cell in a laboratory flask to an economically sound industrial process. Though "genetic engineering" has gained wide currency, the scarcity of biological engineers appears as a serious constraint to the expansion of industrial fermentations even in developed countries.

Chemical engineers are generally more experienced in the processing and handling of inorganic and organic chemicals than with biological materials. Consequently scale-up from the laboratory through pilot plant to manufacture has proceeded more empirically than scientifically and even large companies have made costly errors. In confidence we learned how one of Europe's largest distillers built a multi-million dollar facility for a new industrial fermentation process which in less than two years had to be scrapped: the construction materials used were found to be totally incompatible with the products of the fermentation.

Each fermentation presents its own unique set of difficulties, especially in scaling up from glass laboratory vessels to the materials of construction and containment used on a large scale. Maintaining aseptic conditions, optimum temperatures, mixing, aeration and media composition increase in complexity as processes increase in batch size. Particularly complex are the transitions from laboratory batch to continuous flow processes using immobilized cells or enzymes. All fermentations are exothermic: they generate heat. As the scale increases more demanding are the difficulties of heat transfer and temperature control.
Fermentations take place in relatively dilute solutions, consequently large quantities of pure uncontaminated water are essential. Furthermore, the desired end products must be extracted and isolated from the microbiological biomass and the other extraneous metabolites and by-products present. As scale-up progresses the processes of extraction, purification and protection from contamination and change increase considerably.

All microbial species will mutate. Fermentation industries must maintain pure cultures whose metabolic processes and products remain constant and unchanged by unwanted mutations.

Human Health Products

Not surprisingly in the U.S.A. and among aging and wealthy societies elsewhere, interest and investment has been greatest in pursuit of drugs to control cancer, cardiovascular diseases, diabetes and other maladies that afflict the aged. Several biological research institutes were spawned out of university departments to generate such drugs from genetically transformed microorganisms. During their early years these institutes benefitted from a flood of investment, more than $2 B of private money being invested during their first six years. In fact, in the first hour that Genentech stock was offered on the NASDAQ over-the-counter exchange in New York the share price jumped to more than $88 per share. By mid-1984 the Genentech share price was down below $29. Similar share price collapses were experienced by other biotechnology companies while several disappeared into bankruptcy. Of close to 400 new "genetic engineering" companies launched in the USA less than 25 have survived.

These "biotechnology" companies which at first promised rapid rich rewards from drug research now find it increasingly difficult to raise money for projects which become ever increasingly expensive and take longer than originally forecast to reach commercial fruition. Consequently, most of the survivors have now integrated with or are controlled by large established pharmaceutical and chemical corporations, companies with experience and facilities for manufacture and marketing.

Their experience offers several important lessons. Unlike some of the more widely publicized successes in electronics development, successful research and development to generate new pharmaceuticals is not accomplished by two scientists working in a garage. Given the state of knowledge, the successful development, manufacture and marketing of such biologicals requires large investments of risk capital, sizeable teams of experienced research and development workers together with expensive and well-equipped facilities. An experienced Canadian company engaged in vaccine research and development considers a research team of between 18 and 20 scientists the minimum necessary. Though several hundred million dollars have already been spent on genetically reorganized microorganisms to produce such substances as interferons, companies in the know suggest that for every dollar spent on research between $10 and $20 will need to be invested in the downstream activities: for scale-up, clinical testing, packaging, marketing, quality control, distribution and monitoring. Most important, the original
biological research laboratories referred to above suffered from a lack of integration with facilities for manufacturing, sale and distribution. Thus their research was deprived of the essential constant feedback from the factory floor, the market place, and from the monitoring of clinical responses. It seems evident therefore that such applied research is not best carried out in institutions isolated from well established manufacturing and marketing organizations.

Some of the popular literature tends to suggest that once the desired foreign gene is integrated into the fermenting bacteria's DNA, it will infallibly express itself. Appendix II outlines the Canadian experience with the gene for human insulin introduced into E. coli. The fermentation process delivers only an insulin precursor which after extraction from the fermentor, requires 12 to 15 subsequent chemical modifications before becoming clinically potent. To reach the ultimate state of satisfactory manufacture, reliable biochemical potency, safe and effective distribution will probably take about 10 years. By that time it is conceivable that the techniques of implanting encapsulated Langerhans islets (the pancreatic cells that generate insulin) in bodies of diabetics will have been elaborated. From these implanted capsules insulin will be liberated in response to body demand and will not have to be injected interparenterally.

Discussions with Canadian fine chemical manufacturers suggest that, at least until the end of the century, North American meat packers can supply sufficient pancreatic insulin to satisfy all foreseeable needs. The same is true of the sulphated polysaccharide heparin used as a blood anticoagulant and also of several other medically important substances such as steroidal hormones now produced by fermentation of animal cholesterol. Thus before pursuing a recombinant DNA route, it is wise to study carefully alternative and established technologies to achieve the same ends.

Given the enormous quantities of animal by-products discarded and wasted in the abattoirs of many developing countries, a careful comparative analysis of the economies of adapting existing technologies for extracting fine chemicals from animal organs before embarking upon synthesis by genetically altered microorganisms would seem to be worthwhile.

Most important is the need fully to assess the total resources required safely and effectively to deliver health products to the points of end use and where necessary to establish and strengthen the health services needed to do so. Rather than beginning with research and development, some developing countries appear to regard as a safer route, the importation of vaccines and other drugs in bulk under licence. With the assistance of the licensor they first establish facilities and train people for repackaging, the maintenance of quality control and distribution. Once the volume is large enough the licence can be extended to manufacture of the product. When the manufacturing and distribution facilities are well in place, markets established, people trained for all stages of production, quality control, marketing and monitoring, consideration can be given to establishing research facilities to produce new products to pass through the established manufacturing and marketing facilities. To start at the research end of the spectrum, to devote several years developing products
for which no markets have been defined, for which no manufacturing facilities exist, for which no organized system of trained and experienced human resources is in place, bears a very high risk and unpredictably large capital and operating investments. As stated above, even where such facilities exist, complete with the resources needed for adequate pharmacological, clinical testing and monitoring, the time and financial investments in the downstream often exceed ten times the outlays on research.

Veterinary Medicine

Genetically altered microorganisms offer considerable hope of new and more effective means for the diagnosis and control of important animal diseases. However some of the early promises of quick solutions to long standing difficulties now appear more remote. Elimination of trypanosomiasis would permit an increase of over 220 million cattle equivalent annually to 1.5 million tonnes of meat in the tsetse stricken areas of Africa. Monoclonal antibodies are being employed to identify specific antigens carried on the surfaces of the infecting trypanosomes, the pathogenic protozoa transmitted by the tsetse fly which lead to the anaemia and debilitation typical of trypanosomiasis. These trypanosome antigens consist of dense glyco-proteins. Unfortunately for veterinarians, as fast as antibodies are generated to react with each identified trypanosome antigen, the pathogen synthesizes a new or modified antigen. Several hundred genes seems to be involved in synthesizing these glyco protein antigens, each one being expressed as a modified antigen as each new antibody is produced. Thus, in spite of the elegance of these new diagnostic techniques, the pathogen appears to possess extraordinary resources by which to defend itself and to keep ahead of each new antibody by generating yet another different antigen.

Nevertheless the animal health products industries will probably become among the principal beneficiaries of fermentations using genetically transformed microorganisms. Given the size of the animal populations to be immunized (animals are slaughtered and each new-born creature must be immunized), the scale of animal vaccine production will likely exceed most human vaccines.

Also though well established, tried and tested animal vaccines are manufactured and distributed for administration by farmers in a number of developing countries, many do not possess veterinary organizations and industries fully equipped with the research, diagnostic, effective evaluation and delivery systems essential to the development, manufacture, safe and efficient distribution of new vaccines. Furthermore even in developed countries, the state of technological development and bioengineering skills are probably inadequate to construct and operate the scale of fermentations needed in the future.
As is the case with human health products, government planning agencies in a number of countries would welcome advice on the total assembly of resources necessary to establish safe and effective development, manufacture, distribution and monitoring of animal vaccines.

Agricultural Applications

Appendix III describes briefly the possible applications of plant cell and tissue culture in plant breeding. In this section only two aspects of industrial fermentation will receive brief comment: (a) biological pesticides; (b) nitrogen fixation.

Biological Pesticides

Biological pesticides embrace naturally occurring or genetically restructured organisms that are specifically pathogenic to important pests, parasites or weeds. One of the most publicized was the deliberate spread of the fatal viral disease myxomatosis among wild rabbits.

The potential spectrum of biological pesticides, which also include toxic substances of microbial or plant origin, is enormous and largely unexplored. One need not dwell upon the safety hazards that biological pesticides may present to humans, animals and crops. What in the laboratory may appear species or strain specific may, when released, prove more widely pervasive.

Some of the larger agrochemical companies are seeking in vitro plant tissue culture techniques by which to isolate genotypes of cereals, other food and forage crops that are resistant to the herbicides they manufacture. However past experience shows that techniques which appear effective in the laboratory may give quite different results in farmers' fields. Consequently, any move to employ biological pesticides should be made with extreme caution and particular care should be taken to prevent manufacturers in developed countries offering for sale biological pesticides to developing countries which lack adequate means to control and monitor their use. It is not unknown for agencies in technologically advanced countries to offer to less developed nations products forbidden for use in their home territories.

Nitrogen Fixation

As stated elsewhere, the practicality of economically transferring nitrogen fixing genes from legumes to cereals appears remote in the near term. There may be more immediate scope for producing improved strains of Rhizobia by DNA transfers. Nevertheless, according to an experienced legume breeder, his international research centre places a higher priority upon collecting and evaluating the many existing yet unexamined native strains of Rhizobia, of improving techniques for their storage and application, and of identifying those environments most congenial to these natural yet at present unevaluated strains. This scientist emphasises the necessity of any genetic transformation of Rhizobia or mycorrhizal fungi being conducted in close cooperation with experienced plant breeders, agronomists and soil scientists.
Food Fermentations

Appendix IV describes some of the many foods traditionally processed by fermentation. Many have progressed from kitchen crafts to large and small scale manufacturing technologies; from batch fermentations to continuous processes. The scale of these industries and the demand for appropriate skills sustain many university departments which offer degrees in food science and food engineering. The numbers who graduate in food science appear greatly to exceed the numbers of biological and food engineers.

In this appendix only one aspect of food fermentation will be considered: protein from microbial sources.

Most food legislation is predicated on the assumption that the bulk of what is consumed is derived from plant and animal materials: cereals, legumes, roots, fruits, vegetables, fish, carcass and organ meats which have been eaten over many generations. More recent legislation relating to food additives assumes them to be substances of known and analysable composition added in relatively small proportions to fulfill a clear, specific and desirable purpose. It is a sine qua non that extensive toxicity trials have demonstrated their safety within the prescribed limits of use. A working group of senior food legislators convened at IDRC concluded that no reliable methods exist by which to determine the safety of totally novel foods intended for repeated consumption as major components of human diets. Such would include microbial protein derived from large scale fermentations of microorganisms, whether natural or genetically modified, for which no previous systematically recorded history of human consumption is in existence. Therefore, before totally novel foods of microbial origin can be admitted to replace conventional macro-ingredients in human diets, completely new testing protocols will have to be devised and put in place. The working group emphasized that the wealthier nations through aid programs should not press upon developing countries what they themselves would consider unacceptable.

The working group also questioned the desirability of feeding sizeable quantities of novel microbial protein to farm animals used for meat and milk production since only long term feeding of the resulting carcass meats or milk products would bring to light any undesirable changes in nutritional value or potential toxicity.

Human Resources

Industrial fermentations can generate many products, diverse in nature, composition and end use (see Table I). Though many fermentation technologies share common conditions and constraints, each is in itself unique and influenced by many factors in addition to those mentioned above. Naturally occurring biological materials vary greatly in composition, even within species; all are susceptible to change following harvest or
slaughter. Research, development and manufacture call for a broad spectrum of scientific and technological skills and experience. In particularly short supply in most countries are biological engineers: people with a broad knowledge of chemical engineering (reactor design; properties of materials; applied thermodynamics; heat and fluid flow), biochemistry and microbiology. In addition to the sizeable cadres of microbiologists, biochemists and biological engineers needed by the expanding fermentation industries, extremely competent organic chemists and analysts are essential to ensure that each fermentation delivers substances of the quality and composition intended. Organic and physical chemists are necessary since many industrial fermentations generate products which require several modifications to their chemical composition before delivery to the market. Additional extensive ranges of expertise are needed in fermentations which generate pharmaceutical and veterinary products as is described in Appendix II.

Market Identification

Recent experience has demonstrated the hazards attendant upon starting a research program before first defining the market to be served and the resources needed to service the market defined. The products of industrial fermentation serve a vast array of markets. The most successful biotechnology enterprises are those that have first identified and thoroughly analyzed their target markets before developing products and technologies to serve these markets. They have also been highly selective and have confined themselves to a product range sufficiently narrow to be manageable and controllable. To sustain an economic level of production many manufacturers find it necessary to export some of their products beyond their national borders. Japanese manufacturers, particularly successful as suppliers of antibiotics, enzymes and food flavours, appear to charge much of their overhead costs to their local Japanese customers thus making their export prices difficult to compete with.

It cannot be over emphasized, particularly to developing nations, that investment in research and development, unguided by professional market research, is more likely to lead to frustration than to financial success. Consequently, one must question the wisdom of creating national institutes of biotechnology pursuing research and development over a broad spectrum of biological materials and processes; institutes devoted to applied research in isolation from and out of context with established means of manufacture, marketing and distribution. The bringing together of widely diverse applied biological research endeavours under one roof offers fewer advantages than ensuring intimate integration of research and development with established and experienced means of manufacture, marketing, distribution and monitoring. Given the immensely dynamic and fluid state of fermentation research and technology, all successful research must be in constant contact with and guided by the changing experience and difficulties encountered as the laboratory process is scaled up to the manufacturing level and as the products of manufacture are distributed for practically useful application.
Research conducted in an institute isolated from the rigours of manufacturing and marketing may well be devoted more to what seems intellectually intriguing than what is practically sound and economically feasible. The evidence from the commercial sectors of North America and Europe strongly indicate that a much greater proportion of commercially successful applied research results from a defined market demand pull than from research or technology push.

Appendix V emphasises the need for adequate regulatory systems to protect the safety of all who may be employed in, in contact with or proximity to the products and processes of biotechnology, particularly where pathogenic or potentially pathogenic organisms are involved. Though they differ in pattern and content, most industrially developed nations have well codified and monitored food and drug regulations and industrial safety standards. These are being expanded or modified to cover new processes and products of biotechnology. Many developing countries have neither formulated the legislation nor have adequate means to monitor food and drug manufacture, testing and distribution in a capable manner. It is essential that developing countries be protected from outside persuasion to permit or undertake research development or distribution of biotechnological products which would not be countenanced in the technically more advanced nations.

Finally, it bears repetition that fermentation industries promise an extraordinary range and diversity of useful products for many industries and applications. But to succeed in this challenging and complex field requires more than a research laboratory. It calls for a shrewd and educated analysis of actual, potential and competing markets: a comprehensive quantitative determination of all the resources necessary to deliver products of constant and reliable composition and quality; a sound assessment of the magnitude and period of investment needed before the first product is delivered and before the economic break even point is reached. Above all, it requires patience, persistence and perseverance at all stages of the process. Dramatic biological revolutions are as improbable as green revolutions.
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EDITORIAL NOTE

This paper was written by a senior scientist in a Canadian company that specializes in the development and manufacture of certain vaccines, hormones and related health products.

Though the technical content of the early sections may appear formidable to non-specialists, the paper, particularly in the later sections, contains extremely interesting information on the scale and diversity of the resources needed to establish and operate the facilities essential for the safe and effective development, evaluation, manufacture, distribution and monitoring of the biological products discussed.

Introduction

This presentation discusses research, development, manufacture, distribution and monitoring of vaccines and hormones.

The purpose of the presentation is to outline the minimal resources needed to take a research idea to commercial fruition. The research phase is but one step of many and is usually one of the least expensive. Obviously, there will be considerable differences between the experience in a well-established laboratory in Canada trying to improve present levels of technology, and what is needed in building a new laboratory based upon limited experience in a developing country. Nevertheless, there are more than sufficient similarities to draw some general conclusions that might be of assistance.

Substantial effort is being expended to prepare proteins from yeasts and animal cells rather than bacteria. This is because bacterially-produced proteins are fully denatured, i.e. no disulphide bond formation, and unglycosylated. In yeast, proteins will be glycosylated although not necessarily yielding the particular structures found in animal cells. The technology for growing large quantities of these cells is still developing. At the present time, vaccine manufacturers are the largest users of animal cells utilizing fermentors of up to 500 litres. The major difference between bacterial and animal cell fermentation is the requirement for anchorage dependence by the latter except when transformed.

The following describes briefly some products of importance:

Bacterial Vaccines:

These are of four main types:

i) A number of pathogenic bacteria, e.g. tetanus and diptheria, release toxins which have a devastating effect on surrounding tissue. Antibodies to the inactivated toxin (toxoid) are known to prevent
infection. Fermentation conditions have been established under which the production of toxin is favoured. After purification, the toxin is chemically treated to cause inactivation.

ii) The whole organism, inactivated by heat or chemicals such as formaldehyde, elicits an antibody response that is protective. The main human vaccine still prepared this way is that for prevention of pertussis (whooping cough). A number of vaccines for prevention of diseases in animals and fish are also prepared this way.

iii) New vaccines currently in the approvals process have only protein antigens present, i.e. all extraneous nucleic acids and lipids have been removed. An example is that of the subunit pertussis vaccine which is composed of two principal components. While these vaccines are expected to be safer, the cost of manufacture will, of necessity, be substantially above that of the currently licensed products.

iv) Polysaccharide vaccines. Encapsulated bacteria such as Neisseria meningitidis and Haemophilus influenzae, the principal agents causing meningitis are poorly antigenic. The isolated polysaccharide linked to a strong protein antigen such as diphtheria toxoid is changed from a weak to a strong antigen providing long-term protection.

In all cases, large quantities of bacteria are grown in fermentors. However, fermentation conditions for each type of product differ substantially. In some cases, fermentors of different design are required.

For our purposes, fermentors of up to 500 litres are sufficient. While larger units could be used, world demand seldom warrants it. It is important to maintain a regular production schedule due to the high cost of inventories and difficulties of obtaining suitably trained staff.

Viral Vaccines:

These are of four types:

i) Whole virion: After virus growth, the virus is chemically treated to inactivate nucleic acid and prevent further replication. Examples of this process include vaccines for polio, rabies and influenza.

ii) Split vaccines: Treatment of virus with enzymes or detergents inactivates them and dissociate the proteins. While the level of protection obtained may not be as high as with whole virions, side effects are often significantly reduced. An example is that of influenza vaccine.

iii) Single antigen vaccines from viruses: A few years ago the hepatitis B virus was separated from the blood of infected persons. Following inactivation, this product was used as a vaccine. The extremely high cost of the product has precluded its use in all but very high risk groups in selected countries.
iv) Genetically transformed vaccines: The hepatitis B surface antigen is the only viral vaccine prepared in this way that is approaching regulatory approval. The antigen is expressed in yeast or animal cells in a fully assembled form. The protein is sufficiently large to be an effective antigen. Small proteins are readily prepared but are usually non-immunogenic. The cost of the product prepared this way is substantially below that of the vaccine prepared from infected blood.

Hormones:

Human insulin is presented as a model of this group of biologicals.

Insulin and human serum albumin are exceptions among biological products in that the dose is considerably above the microgram level. With insulin, the normal dose is just about one milligram while albumin is given in gram quantities. Human insulin will be discussed in more detail later. Monoclonal Antibodies:

Only in the past few months have monoclonal antibody products been approved for human use in Canada.

Therapeutic use of murine monoclonals will be limited; the scope is far greater for human monoclonals. The ultimate method of production is one that uses non-anchorage dependent cell growth. The methods that have been utilized for large-scale production of monoclonals are:

- Placing hybridomas within a semi-permeable capsule permitting the free transit of nutrients and metabolites, but not the antibodies. The cells replicate to fill the capsule. Capsules are easily harvested and washed to remove contaminants before opening and separating cells and supernatant fluid containing antibodies. This method has been patented by Damon Laboratories.

- Direct expansion of the murine ascites fluid method. This method is tedious and expensive leading to numerous possible quality assurance problems.

- Celltech of Great Britain have concentrated on large-scale fermentation methods. They have utilized air-flow fermentors handling over 100 litres of culture.

- An interesting method has been developed by Bio-Response of California. Hybridomas are cultured in containers supplied directly with lymph drawn from a living calf. This permits the recreation of
an environment similar to that in the body in which mass production of monoclonal antibodies can take place.

Footnote: Most non-cancerous mammalian cells cannot be grown in suspended culture; they require to be "anchored" upon a solid surface, frequently glass. Such cells are said to be "anchorage dependent". Cells which can grow suspended in a liquid medium are said to be "non-anchorage dependent".

Hybridomas produced to date are not as vigorous or sturdy as mouse hybridomas and significant quantities are difficult to obtain. Considerable research is being conducted on this subject and major advances can be expected in the next few years.

Regulatory Issues

In North America, Europe and Japan, human biological products are subject to very strict regulatory control. Most developing countries will eventually require adherence to WHO regulations which are comparable to those of the U.S.A.

These regulations are aimed at defining acceptable criteria for all biologicals and of establishing that appropriate controls are applied at the various production stages. More detail will be provided later in this paper but some comments are perhaps needed regarding the regulation of products from recombinant DNA or monoclonal antibodies. One of the most crucial stages from a regulatory viewpoint is the definition of seed cultures, the starting point for each product lot. With plasmids, sequencing of nucleotides is relatively straightforward permitting unambiguous characterization. Bacteria and hybridomas, on the other hand, can be characterized only in terms of certain surface antigens and excreted products. The definition of cell seeds to ensure that each vial is identical over long periods is far more complex. There are numerous other examples that further illustrate the complexity of the problem.

What follows is a description of the many constraints that exist when a product is taken from the concept stage to the approval for marketing of a biological product that will be used for disease prevention. A parallel can be drawn from this class of medicine to therapeutics and other products of high technology utilizing biological processes. It must be emphasized that the duration and cost of the research stage is one of the shortest and least expensive of the many steps involved. (See Table 2).

The Research Stage

The ability to be successful in research will obviously vary widely both with the quality of the scientists involved and of their management. It is assumed for this paper that the laboratory will be staffed by competent professional scientists since truly outstanding individuals are very rare. The actual level of staffing will vary with the intensity of effort required, but a number of generalizations are possible.
Marie and Pierre Curie achieved much in a crumbling shed with minimal equipment. However both suffered serious health problems because of the then unknown dangers of their work. Biotechnology requires facilities substantially better. Work with bacteria, yeast and animal cells requires addressing the safety issue in terms of facilities - safety for the organisms, the workers and the community. The integrity of fermentations and bacterial strains is maintained by sound sterile techniques in well serviced facilities. Should work be done with viruses (eg. rabies), the level of containment becomes particularly important to those working inside and outside the laboratory. At the risk of stating the obvious, the laboratory must be large enough to permit staff to operate safely and to contain the necessary equipment. It is not possible to have the research and development equipment in the same laboratory; the differences in scale and construction are too great.

Staff requirements

Assuming that any biotechnological program has three distinct phases, one can generalize about staffing requirements:

Genetics: Whether complex DNA manipulation or only strain selection is planned, a microbial geneticist is necessary. In the former case, additional staff will be needed for the many cloning, selection and characterization steps. There is a great need to establish a critical mass of at least six scientists to allow suitable exchanges of ideas. These scientists will require the support of skilled technicians.

Fermentation: Any microbial product must ultimately be propagated in quantity. The conditions under which large quantities are produced are defined by the fermentation staff. Suitably qualified fermentation engineers are difficult to locate and more difficult to hire, particularly in North America where they command high salaries.

Downstream Processing: The process whereby the expressed protein is converted to the form required is frequently a long and tedious process requiring highly skilled protein chemists and expensive, highly specialized equipment. The staff in this area require skills different from those in the genetics and fermentation areas.

   Biotechnology requires expensive, complex equipment such as ultracentrifuges, oligonucleotide synthesizers (gene machines), High Performance Liquid Chromatography (HPLC) apparatus, fermentors, and protein sequenators. The cost of this equipment will amount to at least $500,000 for a well-equipped laboratory. The cost of maintaining and constantly upgrading the equipment is also high. Even in Toronto some difficulty is experienced in obtaining rapid repair service from manufacturers. Located well away from the manufacturer, the equipment will have substantial downtime and even higher maintenance costs. Most equipment is too complex for more than routine maintenance by local staff.
Recombinant DNA uses a great many chemicals and enzymes. The latter are available from several suppliers but are usually shipped by air in small quantities. Their high cost is compounded only by the cost of shipping. This Toronto research facility employing 18 people uses about $15-20,000 of supplies each month. Shipping costs will be higher at greater distances. Considerable planning will be needed to arrange a reasonable delivery schedule of reagents to forestall even higher costs associated with rush shipments.

**Animal Testing Facilities:** Most biologicals require assessment of safety and efficacy in an animal test model. The most common animals used are rabbits, rats, guinea pigs and, occasionally, monkeys, although these latter animals are not always readily available. For a test to be meaningful the animals must be as nearly identical as possible. There are two ways of obtaining these animals, purchase or breeding, both associated with very considerable costs. To breed animals, a facility must be assembled that ensures the genetic integrity of each separate strain and the safety of the animals. Inadvertent introduction of disease into a breeding colony can cause immeasurable damage, often undetected for months. Staff may transmit pathogens from one strain to another. Care should be taken to ensure that animals remain properly segregated within the facilities. Most modern breeding colonies deliver infant animals by caesarian section to maintain freedom from infections during normal delivery. If the animals are to be supplied by a commercial breeder located in Europe or North America, the logistics of transporting animals must be considered. Animals cannot be shipped as normal cargo, nor can they be left unattended in the heat or cold of an airport on route. The transport costs of such an operation are extremely high.

It is difficult to put a figure on the size of animal colony needed. In fact, the size will fluctuate with actual requirements. For routine safety testing of known products the time and number of animals required is easy to predict. The timing and resources needed for new products of research is far more difficult to predict. Animal breeding calls for long-term planning taking into account gestation periods and breeding cycles. Test animals cannot be produced at a moment's notice!

Any product destined for human use, particularly those for medical or pharmaceutical use, must be fully tested in animals before any human testing is allowed.

The high costs associated with the research phase will actually seem small compared to the costs associated with bringing the product to market. Once success has been achieved in proving the research concept and that small quantities of the product produced in the laboratory demonstrate the biological effect required, the research staff, together with others, can then begin the complex task of scaling up the process and making it economic. Using examples of products being studied in one laboratory, an attempt will be made to show the resources required for each of the steps from development through to marketing and sale.
Research and Development

The gene for human insulin was obtained by synthesis while that for rabies glycoprotein was obtained from a cDNA copy of the viral RNA, a process taking many man-months of intensive work. The genes were sequenced to prove identity with published nucleotide or consistency with protein sequences and inserted into plasmids modified to contain the appropriate selection markers (in this case antibiotic resistance genes), an origin of replication, the promoter region and some of the coding region of a major inducible protein, e.g. B-galactosidase, followed by the gene of interest and the appropriate termination region.

Bacterial colonies containing the plasmid of interest were selected utilizing the antibiotic resistance characteristics of the plasmid. These were grown further for characterization and additional sequencing. Having established that the construction was correct, sufficient bacteria were grown to permit isolation of sufficient protein for characterization.

In the case of human insulin, the precursor protein was produced as an insoluble aggregate of a B-galactosidase leader (289 amino acids) and proinsulin (86 amino acids). Following bacterial growth, cells were collected by centrifugation or filtration and washed. Disruption of the cells gave the insoluble precursor contaminated by only a relatively small amount of cell-associated protein. This precursor could then be cleaved to proinsulin, refolded (in E. coli, no disulphide bonds are formed) and converted to insulin (51 amino acids).

Subsequent work permitted the use of multiple copies of proinsulin behind a 9 amino acid leader of B-galactosidase. The proinsulin units were linked with small peptides which could be removed upon enzymatic cleavage to insulin.

The expression of rabies glycoprotein was similar except that yields were lower, and the necessity of reforming correctly 22 disulphide bonds has not proven economical, and other methods are being attempted.

The genetic engineering and the associated fermentation work was conducted by a total of 18 people of whom 6 were at the doctoral level. This is the smallest group possible for a program of this complexity. Minimum staffing necessary includes a project leader, who has overall responsibility, and team leaders for genetics, protein chemistry and cell growth. Other staff report to these persons to conduct the day-to-day running of the laboratory. As described above, considerable attention must be paid to the regulatory issues, particularly pertaining to definition of the seed culture.

Scale-up

Having established that the genetic construction is correct and that reasonable quantities are produced, the simple process of taking this from 100 ml flasks to 100 litre fermentors begins. Ideally this process is
simple, works the first time and gives product yields at least equivalent to that obtained in small-scale experiments. In practice, this is a highly frustrating process that invariably takes longer than planned, costs substantially more money and yields less product than expected. These difficulties can be minimized by experienced planning and careful management of the processes.

It is rarely possible to move directly from a laboratory flask to a full-scale fermentor, particularly for a product required in quantities as large as human insulin. In this case a 20 litre fermentor was used as an intermediate to evaluate fermentation conditions. Among the conditions to be established are medium composition, determination of which components are consumed, inoculum, growth parameters, pH and dissolved oxygen requirement, harvest time to maximize product yield. This latter is a combination of total biomass produced and the level of expression. Conditions must be found that minimize loss or mutation of the inserted plasmid. The conditions must be fully defined so that scale-up from 20 to 100 litres or more permits full replication of conditions within the fermentors.

This is a time-consuming and expensive operation. When large quantities of the product are needed, as is the case with insulin, some form of continuous or semi-continuous fermentation is needed. A two-stage fermentation process complicates the evaluation of fermentor parameters in many ways. The primary fermentor is used for bacterial growth (biomass) and the secondary unit for expression of products. Accurate control of these complementary components is essential.

Downstream Processing

Removal of cells is fairly straightforward and centrifugation (with its attendant aerosols) has given way to filtration. Closed units are available that allow total separation of cells from supernatants. The necessary cassettes are expensive but reusable. To produce human insulin requires the cells, while other vaccines require the contents of the supernatant.

The cells are broken open. On a laboratory scale this is performed with a homogenizer whose action may be complemented by lysozyme acting on the cell walls. In large quantity, it is often best to use special equipment such as a bead mill. The insoluble precursor is dissolved in formic acid. The differences in using 25 ml formic acid and 25 litres are significant. Fume hoods that allow 25 litres of reagent to be used and not readily available. The solution is treated with cyanogen bromide for several hours to cleave the protein at the methionine residue inserted just ahead of the proinsulin gene. Solvent exchange in laboratory scale is easily conducted in dialysis tubing. For large quantities one usually uses ultrafiltration units. But membranes that can handle 75% formic acid are not obtainable. Chromatography is also quite different when moving from the millilitre to litre level. Conditions to handle each of these steps
must be individually worked out. The refolding of proinsulin is fairly straightforward being complicated only by the incomplete reaction requiring recycling of incorrectly folded material. The conversion of proinsulin to insulin is achieved by the addition of enzymes to the proinsulin solution. However, when working in large quantities it is often not possible to stop the enzymic activity at the correct time and further proteolysis occurs causing substantially decreased yields of insulin. For some reactions immobilized enzymes offer hope of greater efficiency but the techniques still require a good deal of development.

When dealing with products released into the culture supernatant as is the case with hepatitis B vaccine or interferon, purification is greatly simplified. Affinity chromatography on insolubilized antibodies will effect substantial purification and concentration since these proteins are needed only in small quantity and require no chemical modification.

The safety aspects of process scale-up are especially serious when working with pathogens. Equipment on the scale needed is not designed to fit within containment hoods; the building is in effect the hood, and the production staff must work within it. Pilot plant staff are not necessarily the same people involved in the basic research. The former are usually persons more used to solving the many practical problems arising daily. Experienced persons are difficult to find and are in high demand.

The scale-up of products such as human insulin cannot be conducted by fewer than six people of whom at least two have post-graduate training in fermentation and protein chemistry. Other than support staff, all members of these groups would be graduates. In dealing with products not requiring such extensive purification processes, a team of four is probably minimal. The purpose of all scale-up work is to define the processes needed for production. In dealing with biologicals this implies the manufacture of clinical trial lots, the trial itself and finally the regulatory approval process. Before even starting these steps, considerable additional information is needed about both the process and the product.

- Do the facilities meet regulatory standards, either Good Laboratory Practices (GLP) or Good Manufacturing Practices (GMP)? This is a complex question requiring detailed review of facilities and procedures. It is beyond the scope of this presentation to go into the details of GMP. However, a few issues will be discussed. The standards for Water for Injection is defined in various pharmacopoeas. Maintaining the standard of water at or above this level is an expensive, energy-consuming process. The testing necessary to prove water suitable is also expensive. Yet water is the major constituent of the fermentation and downstream processing steps. The other raw materials used must also be defined, which implies that they be tested to prove they are as labelled. It will also be necessary to test equipment such as autoclaves to ensure satisfactory operation. GMP is not a codified list of tests, but an attitude which, if followed, will greatly reduce the possibility of a compromised product.
Has the product been fully defined in terms of safety and efficacy? A series of laboratory and animal tests are needed to establish, as far as possible, the full range of biological effects and to determine those parameters acceptable for the product and its intermediates. These tests should be consistent wherever possible with published regulations of the World Health Organization or the U.S. Code of Federal Regulations, and should be performed by a separate quality control department.

For a number of products administered to man, a series of toxicology tests are required. These may range from acute toxicity tests taking only a few months in two or three animal species to carcinogenicity testing which may take two years. Such testing is conducted in at least two species. The cost of toxicology testing is prohibitive and may exceed $1 M for each compound.

Has the production process been sufficiently defined to permit exact replication? Clinical trials are conducted on material prepared from successive lots. One aspect reviewed in the approval process is the manufacturer's ability to establish consistent production although the clinical lots may be made in equipment smaller than that used in full-scale production. The processes used, however, must differ only in scale or a new trial may be necessary.

Clinical Trials

The purpose of clinical trials is to prove safety, immunogenicity and/or efficacy in the population for which the product is intended. The U.S. Food and Drug Association has established four levels of clinical evaluation:

i) **Phase I**: Initial safety in a normal population. For a new pediatric vaccine this may represent an initial evaluation in a small number of adult volunteers. For an improved vaccine, these studies may involve administration of a single dose to vaccinated adults. For human insulin, a Phase I study would likely involve administration of small quantities of insulin to non-diabetic volunteers.

ii) **Phase II**: Limited safety and efficacy studies conducted in the target population. The actual numbers of persons participating in the trial is a matter for negotiation between the manufacturer and the regulatory authorities.

iii) **Phase III**: Granted that the Phase II studies are satisfactory, approval may be given to expand these studies into a far larger target group. These studies may involve thousands of administrations of the product and will usually, in the case of a vaccine, involve a full course of therapy. For many childhood vaccines this extends over 18 months. Based on the results of these trials, approval can be sought for a product licence (New Drug Application, NDA). In other cases, a trial designed to prove product efficacy is required.
iv) **Phase IV:** In some cases approval is given for product launch conditional upon post-marketing surveillance of the product. Depending on the nature of the product, this may cover an extended period.

All clinical trials are subject to the Helsinki Convention which requires informed consent from participants. Even with strict adherence to the terms of the Convention, a number of ethical questions remain:

- Can the testing of a new and possibly inferior product be conducted when a superior product is available? This could possibly occur with rabies vaccines where the neural tissue vaccine is substantially cheaper than that prepared in human diploid cells.

- Will the careful monitoring of the clinical trial population produce an effect not seen in the general population? This might be most noticeable in controls giving a lower than anticipated attack rate.

- How well is it possible to control the trial? Ideally, the best comparison for an efficacy trial is a treated against an untreated group. This is seldom possible. The net result of this dilemma is to conduct a trial to demonstrate an improved effect over an existing product, a procedure requiring a far larger trial population and higher cost.

- The use of an actual challenge with disease-causing organisms is readily done in animals, but in man it poses quite a problem. It has been used in assessing treatment and prevention of diseases such as influenza and gonorrhoea, but is quite impossible with some of the more serious diseases such as rabies.

- Should the trial be completed when early data show the product to be highly effective? The alteration of the trial protocol could well lead to incomplete assessment of some of the parameters.

The cost of these clinical trials is very significant and will vary with the location of the trial. In areas where the delivery of health care is centralized (i.e. socialized), much of the cost of the trial becomes absorbed within the system. In other areas, trial costs are listed as actual payments to physicians and clinics. The trial must be under the direction of a physician, preferably an employee of the manufacturer. His principal duties are to plan the trial, arrange the physicians to do the actual selection of patients to specified criteria and to administer the test materials, monitor the progress of the trial, arrange the analysis of any body fluids, eg. blood, for evidence of product efficacy and to analyze the trial data prior to preparing a final report for inclusion in the NDA.

For clinical assessment, 3-5 product lots are used. These lots establish consistency of manufacture in addition to providing material prior to initiation of production. These product lots are subject to the
same level of testing expected for the licensed product. Table 1 lists tests to be conducted on clinical lots of a bacterial vaccine, a viral vaccine and human insulin. The regulatory authorities may request additional testing or to repeat testing in their own facilities.

**Production**

The current construction costs of a plant to manufacture both bacterial and viral vaccines in a developing country is between US $500-600 per m². Such a vaccine plant was recently constructed in North Africa and occupies 10,000 m² and consists of facilities for:

- Washing and sterilizing
- Media preparation
- Storage of seed cultures
- Growth of cell, virus and bacterial seeds
- Separate fermentation and cell culture areas having the appropriate biological containment
- Downstream processing facilities
- Filling and packaging of product
- Quality control testing
- Administration

This plant also has the necessary equipment for air and water purification, air conditioning and refrigeration. The actual production equipment necessary will cost another U.S. $6-8 M and will include items such as back-up generators, fermentors and monitoring equipment and filling machines. As with all major equipment, maintenance costs will be high since spare parts and expertise will be maintained only in a few major centres.

Since the production process may take many months (in one known instance the product takes 14 months from start to final release), careful production planning and production cycling is necessary. When switching from preparation of one product to another, the facilities have to be exhaustively cleaned to assure no cross-contamination of other organisms, e.g. pertussis and diphtheria. This leads to the necessity of storing sufficient concentrated intermediate product. However, experiments will have to be performed to establish the most favourable storage conditions and the maximum storage time. Stability studies will also have to be done on the final formulated product to determine how long it remains viable and under which conditions. These tests will continue for several years. Most biological products are kept at 4-8°C for maximum stability and have a dating period of one year unless proven otherwise.
While it is not necessary to maintain a 24 hour watch on production fermentors, close monitoring of the process is essential at the start. Periodic inspection is all that is necessary for the remainder of the cycle if some form of monitoring is provided by recording instrumentation to prove that the fermentation has proceeded in the anticipated manner.

Considerable thought must be given to the selection of the production manager. He/she should be a qualified microbiologist in addition to being a capable manager. Others involved in production should be microbiologists and chemists although it is difficult to specify actual qualifications. In a major Canadian bacteria plant 32 people are employed, eight of whom have university degrees. This plant produces vaccine in quantities substantially in excess of that to be used by a single country, but may serve as a model for other biotechnology products produced in quantity. The viral facility employs a similar number of people. Suitably trained production staff are not readily available and considerable in-house training will be required.

Filling and Packaging

This process is also a complex one. The product is transferred from holding vessels to vials such that each is identical and sterile. Though automated machinery is available to perform these tasks it requires considerable expertise to maintain it. The equipment itself is expensive and must operate, at least in part, in a sterile environment.

Quality Control Testing

In addition to its role in establishing quality assurance, the Quality Control department must ensure that only the product that meets or exceeds the product standard is released. Thus a series of analyses are required. The release of a product by the head of QC should be independent of production or marketing pressure. The QC department must be staffed by persons able to test the product and to review intelligently the data to determine if release is justified.

Commercial

Some of the particular difficulties associated with vaccine delivery will also occur with other products of biotechnology. It is essential that program administrators, researchers and production personnel retain a clear definition of the end product of the work, the end user and his level of expertise and sophistication and the conditions under which the product will be used. There is no point in requiring a dosage schedule involving complex calculations for a user who cannot understand them. The formulation of the product must be designed for use by the chosen end-user and is obviously governed by technical practicalities. While we would wish to have all vaccines stable for long periods of time at ambient temperature, highly effective by single-dose oral administration providing
lifetime protection, these objectives are rarely achievable and compromise is necessary due to the nature both of the organisms and the immune response of the recipient.

There are a number of cultural aspects that have a significant impact upon product use. In the field of vaccination, this is seen by the fairly good attendance of initial immunization and poor attendance for subsequent administration. There is certainly no inexpensive answer to this question, and short of coercion, there is no simple way of ensuring compliance with immunization schedules. Similar difficulties occur in retraining users to handle the product correctly. It is not uncommon for busy physicians to be unaware of changes to vaccine formulations and methods of administration in spite of the manufacturer's efforts to inform him/her of these changes. Introduction of new product types with complex applications will require widespread retraining. For some products of biotechnology aimed at general use, the problem is compounded by limited literacy. The resolution of these particular issues requires substantial expenditures by the marketing organization to speak to all users.

Sometimes the infrastructure required to maintain the product is not in place. This is best represented by one of the major impediments to the wider application of pediatric vaccination programs in developing countries - lack of a "cold chain". Vaccines, being biological agents, sometimes living viruses, usually retain activity only if maintained at refrigerated temperature. Heating, cooling or freezing (a problem in Canada) results in substantial product deterioration. Vaccines are often needed in remote areas far away from reasonable refrigerated storage. If these products are to be more widely used, the expensive process of establishing suitable storage and transportation facilities must be instituted. Many of the products of biotechnology (not just those for health care) will require careful adherence to storage protocols. Unless a suitable distribution system is in place, there will be no significant commercialization of successful research conducted in laboratories.

Another difficulty with the marketing of biological or biotechnological products is that of cost. In an operation making significant quantities of vaccine, actual production costs are fairly low due to the microgram quantities actually required for immunization; the major costs are associated with filling and packaging, quality control and assurance, marketing, delivery and, particularly in the U.S.A., insurance. Elimination of all profit margins on vaccines sold in developing countries will not significantly decrease the cost of administration of these programs. While some government maintenance of research is both advisable and essential, attempts should be made to have the production facilities largely self-supported by sales. This obviously poses immense problems for management. National interest, particularly in areas with disease problems not common in Europe or North America, may dictate extensive research programs, but these will need funding either from the government of the country affected or from one of the foreign aid organizations. Such aid
will likely be given more readily if it appears that the country concerned has the ability to bring the products of the research to a commercially viable process.

Most vaccines and pharmaceuticals are being produced in a highly competitive environment which has resulted in substantial reductions in price. The quantities of product are frequently enormous permitting significant economies of scale. Unless the local plant has protection, its product will be more expensive. For example, a Canadian firm produces tetanus toxoid in 1000 litre quantities. Each operation, such as testing, filling and packaging is conducted on each 1000 litres. Had they produced 10 x 100 litre lots, these costs would have been 10 times higher. It may be that low level production is necessary if these products are needed to fulfill a national strategy of self-sufficiency, or some other valid reason but the decision must be one taken with full knowledge of the consequences. With suitable facilities in place it may be more useful, initially, to purchase bulk quantities of product from a major producer and repackage to meet local demands.

The costs of marketing are high. An average marketing cost for the first 5-10 years of product life is about 35% of sales for pharmaceuticals, biologicals, agricultural products, animal feeds and cosmetics. In the initial launch period, marketing costs may well exceed total annual sales. This may be true even in countries where all vaccine purchases are made through a central agency. The cost of training of end-users and of establishing the cold chain, for example, must still be borne.

Who is responsible for product liability issues? Administration of biologic agents in vaccination programs is associated with a very low, but significant, incidence of unfortunate and serious reactions. Will there be a government program of liability insurance, or will the manufacturer have to obtain commercial liability insurance?

Management Implications

The management of a program as complex as that of vaccine research, production, and sale is an essential component of the program requiring highly trained and skilled administrators. However the program is structured, development of a new (to the organization) product requires detailed input from several sectors of the organization each having its own set of priorities. A sound central administration is essential to program management. Close liaison is required between the organization developing the product, particularly a vaccine, and the principal users whether it be a government department, a medical association or a user group. Continuing liaison is needed between the groups performing the various tasks (i.e. research, development, clinical, quality control, filling and packaging, marketing and finance departments) to ensure uniformity of product objectives and to expedite the change of the project from one jurisdiction to another. In most cases it is also necessary to maintain liaison with
university researchers both in their own country and elsewhere. It is from these individuals and from product licences from other companies that most new products will derive.

Any future investment in research, any significant breakthrough, or any major push to solve a specific health problem will be successful only if the practical barriers to making the product available on a large scale are lifted. Without research no organization utilizing high technology such as biotechnology can survive for more than a few years. However, there are very few organizations that can generate enough new products to justify starting from the beginning. A self-sufficient organization will conduct research in proportion to its sales, and without a process for commercializing existing products there will be no sales from which to sustain research and development.

Conclusion

Biotechnology is a major area for future products but returns will take a long time to be realized. The process cannot be hurried beyond a certain point and it is the role of management to define this point.

Tables 2 and 3 illustrate the man-days, time and cost projection for each of the phases of the program. The arbitrary cost units bear no relation to any known currency. They are given merely as a means of comparing the relative cost of various steps.
<table>
<thead>
<tr>
<th>TABLE 1. Viral Vaccine Testing</th>
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<tbody>
<tr>
<td><strong>In Process Testing</strong></td>
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<td>Cell culture safety</td>
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<td>Mycobacteria</td>
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<td>Mycoplasma</td>
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<tr>
<td>Adventitious Viruses</td>
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<td>Haemadsorbing Viruses</td>
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<td>Karyology</td>
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<tr>
<td>Potency</td>
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<tr>
<td>Sterility</td>
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<tr>
<td>Identity (of virus type)</td>
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<table>
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<tr>
<th>Bacterial Vaccine Testing</th>
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<tbody>
<tr>
<td><strong>In Process Testing</strong></td>
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<tr>
<td>Seed culture purity</td>
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<tr>
<td>Toxin Lethality</td>
</tr>
<tr>
<td>Opacity/bacterial yield</td>
</tr>
<tr>
<td>Crude toxin sterility</td>
</tr>
<tr>
<td>Purity</td>
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<tr>
<td>Toxicity of toxoid</td>
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<tr>
<td>Toxoid purity</td>
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<td>Toxoid sterility</td>
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<tr>
<th>Biosynthetic Hormone Testing</th>
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<tr>
<td><strong>In Process Testing</strong></td>
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<tr>
<td>Cell viability</td>
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<td>Cell homogeneity</td>
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<td>Biomass Production</td>
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<td>Purity by HPLC</td>
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<td>Electrophoresis</td>
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|                          | **Final Container Testing**                 |
| Pyrogen                   |                                             |
| Sterility                 |                                             |
| Identity (potency)        |                                             |
TABLE 2.
MEAN PROJECT DURATIONS (MONTHS)

<table>
<thead>
<tr>
<th></th>
<th>Research*</th>
<th>Development*</th>
<th>Clinical</th>
<th>Total Man Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Vaccines</td>
<td>26</td>
<td>15</td>
<td>34</td>
<td>325</td>
</tr>
<tr>
<td>Viral Vaccines</td>
<td>38</td>
<td>27</td>
<td>38</td>
<td>385</td>
</tr>
<tr>
<td>Other Products</td>
<td>54</td>
<td>12</td>
<td>18</td>
<td>1030</td>
</tr>
</tbody>
</table>

TABLE 3.
MEAN R&D COST (ARBITRARY UNITS)

<table>
<thead>
<tr>
<th></th>
<th>Research*</th>
<th>Development*</th>
<th>Clinical</th>
<th>Total+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Vaccines</td>
<td>4060 (40%)</td>
<td>2990 (29%)</td>
<td>3130 (31%)</td>
<td>10180</td>
</tr>
<tr>
<td>Viral Vaccines</td>
<td>4285 (40%)</td>
<td>3510 (33%)</td>
<td>2810 (27%)</td>
<td>10605</td>
</tr>
<tr>
<td>Other Products</td>
<td>14530 (68%)</td>
<td>4970 (23%)</td>
<td>1780 (9%)</td>
<td>21280</td>
</tr>
</tbody>
</table>

* Overlap between R&D is considerable.
+ Excludes any costs associated with marketing, licence, capital equipment or facilities.
PLANT BREEDING, CELL AND TISSUE CULTURE

Conventional Plant Breeding

Plant Breeding uses two fundamental operations. The more basic of the two is SELECTION.

Selection involves the identification of the plant or plants which possess the characters being sought. It is necessary that the plant identified can transmit these same characters to its offspring. The breeder can identify the PHENOTYPE, i.e. the appearance of the plant, but must then confirm that the plant has the right GENOTYPE, i.e. that the characters seen are inherited. This must be done by studying its progeny. Progeny rows may be used for this purpose; these consist of the offspring of single plants grown in rows, so that each row can be associated with an individual plant of the previous generation, and therefore reveals its genotype. If all the plants in progeny rows for several generations are uniform, then the original selection was "true breeding", and a "pure line" has been obtained. If the progeny rows are not uniform, plants of the desired type are self-pollinated, and are then themselves grown out as separate progeny rows. This procedure of repeated self-pollination is continued until pure lines of the desired type have been obtained. This usually requires more than six generations to achieve. This process, known as "inbreeding", involves exposing the genetic make-up of the selected plants, taking care to prevent the inclusion of any extraneous genetic factors from outside. Sequential self-pollination generation by generation is the most intensive form of inbreeding. Sometimes this is not possible, e.g. when male and female flowers occur on separate individual plants. Then "sister-brother" mating may be used (sibbing) which achieves the same result over a longer period of time (more generations = more growing seasons).

The second fundamental plant breeding operation is the GENERATION OF VARIABILITY. The desired characters may not be present in the population, or variety, within which the plant breeder is selecting, and so fresh variability must be generated. This may be done by hybridization. Parents are identified, which separately have several desired characters. By crossing the parents together (hybridizing them) a new population is generated, in which a whole spectrum of fresh character combinations occurs.

The next step is selection - choosing the plants which have the desired character combinations, and inbreeding, followed by further selection, until the desired combinations have been "fixed". This sounds very simple, and if one is crossing tall red peas with short white peas in order to obtain short red peas, the process is straightforward. However, when one is dealing with a multiplicity of characters, such a yield, height, straw strength, resistance to several diseases, and quality factors, the problems of getting every character desired into one genotype are very great, and long periods of time are required.
It has been calculated that for at least one plant to occur which contains the combination of 21 characters by which two parents in a cross differed, a population of $4.4 \times 10^{12}$ plants must be grown in the second generation. This is virtually impossible to do, and if one could do it, how does one identify that one plant? Plant breeding is a numbers game with a long time-scale.

The breeding of cross-pollinating (out-crossing) crop plants is based on the same principle of identifying good plants by evaluating their progeny. Inbred lines may be developed, compared, and the best combined together to give hybrids, composites or synthetics. Cross-breeding populations may be improved by mass selection, which involves choosing the best plants in each generation and mixing those together. However, better progress is obtained if the selected plants are inbred for one generation first, the progenies evaluated, and remnant seed from the best progeny rows are combined to make the next stage of the population ($S_1$ testing). Sometimes selected plants may be evaluated by the performance of the progeny of their crosses - to another good plant (full sib), to the bulk population (top crosses) or to a particular line, termed a "tester".

Various plant breeding procedures have been developed to minimize the two problems of population size and time-scale, but as soon as one moves away from working with a few simply inherited, readily identifiable characters, crop improvement becomes a slow process of gradual betterment, the steady ascent of a long slope.

Modern Developments

Evidently, if it were possible to make desirable changes of a few characters in a plant that is already a very good genotype, much time could be saved.

Induced Mutations

Induced mutations have been tried for many years. These aim to bring about changes in individual genes. Once the change has been obtained, inheritance is usually simple, and the desired plant with the one character change can be developed into a new variety quite rapidly. Unfortunately, the process is totally unpredictable: one may bombard plants with radiation or treat with chemical mutagens for many years, yet never obtain the character improvement being sought. It has its place: there have been occasional successes: it may be possible to obtain improved disease resistance in an otherwise excellent variety. However, the place for radiation or chemical mutagen treatments in plant breeding programs is minor. They have not provided the answers to the problems of changing several desired characters over a shorter time-scale than conventional methods.
Plant Cell and Tissue Culture

Flowering plants reproduce themselves sexually by fusion of a male gamete contained in pollen with a female gamete in the ovule. The resulting fertile embryo develops into a seed which when mature and placed in a favourable environment produces a root and shoot from which a new plant grows and in due season flowers and fruits. Self-pollination occurs when an ovule is fertilized by pollen from the same plant. Cross-pollination results when pollen is carried by birds, bees, other creatures, by the wind or the hand of man from one plant to another.

Some plants can reproduce themselves asexually from somatic tissue other than seeds. The process is called vegetative reproduction and can come from bulbs, corms (swollen underground stems), rhizomes (creeping underground stems), tubers and stem cuttings. Cassava is propagated from stem cuttings, bamboo from rhizomes. Any piece of tissue excised from a whole plant and used to regenerate a new plant is called an "explant". Explants may include sizeable cuttings or microscopic pieces of plant tissue.

Recent years have seen considerable progress in the propagation of plants from cell tissue and isolated somatic cells. The explant is placed in a nutrient medium, either a liquid broth or a jelly, which contain various substances which stimulate cell division and proliferation. According to the source of the cells, the composition of the medium and the ambient illumination and temperature, the explant may develop into a callus, a mass of undifferentiated cells, or into an organized pattern of differentiated cells which so arrange themselves, morphogenetically to form a small plant with a shoot and a root. In some instances an undifferentiated callus can be induced to reorganize (differentiate its cells) into a plant by changing the composition of its nutrient medium.

IDRC supported research in which cassava plants, free from infection, were generated by culture of explants from the apical meristems of diseased plants. Meristems being composed of new and proliferating cells are temporarily free from organisms infecting the main body of the plant and therefore meristem culture is being more widely used to generate disease-free material from infected plants. Since some meristem cultures may be preserved by refrigeration, the technique offers a means of storing disease-free germplasm for subsequent propagation, or for transfer from one geographic region to another.

Plant tissue and cell culture involves isolating cells or tissue from live plants and causing them to proliferate under controlled conditions in vitro: in test tubes, flasks or dishes containing a liquid or semi-solid (jelly) nutrient medium. Theoretically a cubic centimeter of plant tissue could produce about one million genetically identical cells each of which could generate a separate cloned plant. In fact, at best, only a small proportion actually yield new plants when cultured. The potential advantages of tissue culture include:
(a) Production of a large number of plants from a single superior plant much more quickly than is possible from seed multiplication;

(b) Generation of disease-free plants from infected parents;

(c) Generation of plants difficult to propagate sexually.

The immediate products of somatic cell or tissue culture include (i) callus (ii) adventitious shoots, (iii) bipolar somatic embryos similar to sexually derived embryos. In practice (ii) and (iii) may be derived successively from (i). (see Fig. 1, 2 and 3). In some instances several changes of media conditions are needed to progress from a callus to a shoot and a root and the subsequent development of other essential organs.

Callus culture can begin with almost any type of explant: from leaves, stems, roots, flowers, pollen, immature seeds, cotyledons, or buds. The reasons why some plant species and media compositions appear more conducive to tissue and cell culture than others is by no means understood, and each new success from cell to callus to plantlet is achieved empirically rather than from a fundamental understanding of the underlying biochemistry and physiology.

Plant cells may also propagate when dispersed and suspended in a liquid nutrient medium. In addition to the culture of intact plant cells, interest is evident in propagation from protoplasts, plant cells from which cell walls have been removed mechanically or biochemically. Protoplasts may be isolated from the primary tissue of plant organs, from callus cultures or from cells suspended in liquid media. Protoplasts may be subjected to experimental manipulations difficult or impossible with intact cells. For example protoplasts can take up foreign genes by micro injection. Protoplasts from different plants may be fused to produce hybrids unattainable by sexual crossing. Nevertheless so far it has proved difficult in many species to regenerate whole healthy plants from protoplasts. Also where whole plants have been derived from intergeneric hybridization many of them have proven sterile and unable to reproduce themselves.

Somatic embryos are similar to sexually derived embryos in that they are bipolar being possessed of a primordial shoot and root. Somatic embryogenesis is particularly desirable from tissue culture since the shoot and root develop simultaneously from a callus or suspension culture. Often, however, the generation of first the shoot then the root have to be stimulated by transfer from one medium to another. The generation of whole healthy plants from somatic embryos is particularly difficult often requiring propagation through successive media each different in composition.
As described earlier, an important objective of plant breeding is to produce pure lines: cultivars which are genetically stable and homozygous having inherited identical chromosomes for any given trait from both parents. To achieve such homozygosity requires that a plant be "selfed" (i.e. fertilized with its own pollen) through several generations: a tedious procedure taking several years for such higher plants as cereal grains.

Each male or female sexual cell (called a gamete) contains only a single set of chromosomes, carrying the genes which control all of the characters displayed by the individual plant. When the male and female are sexually crossed each gamete, male and female, brings to the resultant embryo its complement of chromosomes. Ideally the progeny from the sexual cross would exhibit all the most desirable characters from each parent, characters that would reappear in each subsequent generation. Such does not occur until, as described above, genetic stability has been achieved through repeated self-pollination of the individuals among the progeny that display the most desirable combination of characters.

To achieve stability and uniformity of desirable characters in a plant derived from sexual crossing requires five or six generations of inbreeding by selfing. Since, however, each male gamete contains a single set of chromosomes, if that same set could be induced to double and the cells to proliferate in culture, one would arrive at plants in which genetic uniformity occurred in the first generation. It is possible to double a single set of chromosomes by treatment with a substance called colchicine.

Pollen and Anther Culture

Male sexual cells can be caused to proliferate by pollen and anther culture. Though difficult and entirely empirically developed, considerable progress has been made in generating cell cultures from the pollen and anthers (the male organs which carry the pollen sacs) of several plants. These cells are called haploids in that each contains a single set of chromosomes. By treatment with colchicine each single set of chromosomes can be induced to double, the second set of chromosomes being identical to the first. By control of the conditions of culture several species of economic importance including barley, rice, rye, wheat and rapeseed have progressed to plants from pollen and/or anther culture. In plant breeding by employing pollen or anther culture as a first step, the time required to achieve homozygosity and desired genetic stability can be reduced by several years. Eventually it is conceivable that protoplasts from haploid cells may be fused to generate desirable hybrids.

Somaclonal Variation

In theory each somatic cell is capable of being regenerated into a new whole plant. In practice, each piece of cell tissue that proves amenable to in vitro culture produces a limited number of plants. Originally it was assumed that all plants regenerated from the same piece of tissue would be identical clones. Such is by no means always the case. Many plants arising from callus or suspended cell culture are found to possess markedly different characters each from one another and from the parent plant from which the tissue cultured was derived.
It seems that in progressing from the original organized differentiated state, through a disorganized undifferentiated state, then back to a newly organized differentiated state in the new plants, the chromosomes break up and the genetic code is reassembled in a different sequence pattern in each new plant. Since the process consists of cloning from somatic cells, the results are described as somaclonal variants, each displaying genetically different characters from the original parent.

Since the pattern of the characters displayed by the somaclonal progeny is unpredictable and, at present, uncontrollable, this phenomenon seemed at first undesirable. It has since come to be regarded as a serendipitous benefit in that some variants display desirable characters such as degrees of disease resistance not encountered in naturally occurring germplasm. Because many commercial crops have become very inbred and the collection and characterization of natural germplasm, including wild relatives, is difficult, time consuming and costly, somaclonal variation promises an unanticipated source of genetic diversity for future plant breeding programs.

**In Vitro Selection for Specific Characters**

One of many hoped for benefits from plant cell and tissue culture is in vitro to identify desirable tolerances and resistances. By including in the medium the substance(s) to which tolerance is being sought, it is hoped that the tissue and plantlets that survive will generate progeny that display similar tolerance in farmers' fields. Some such progress is reported for tolerance to salt (saline soils) in rice and oats and to certain herbicides.

The prospect, however, of producing drought tolerant cultivars by tissue culture or by DNA transfers is far from a reality. Tolerance to moisture stress is not a simple phenomenon, nor one that is easily understood. As with many other complex characters much more will need to be discovered about the underlying biochemistry and physiology before any significant progress is made through cell culture or recombinant DNA techniques. It is possible that any introduction of a foreign gene into a higher plant's DNA may disrupt the existing functional system. Without a great deal more laboratory experience and analysis of the total effects of gene transfers it would be unwise to recommend widespread exploitation in higher plants of economic importance. It must be emphasized that progress in plant tissue culture is impressive and what appeared a year ago as speculative and long term now seems soon to be applied. Disease elimination by meristem culture has been described. Pollen and anther culture appear more readily applicable than was thought probable two years ago.

**Genetic Manipulation of Plants**

Because unicellular microbes are comparatively simple in structure, the transfer of foreign genes into the DNA of bacteria is easier than the genetic manipulation of higher plants. Bacterial DNA contains about 5000 genes: a plant genome may contain between 5 million and 50 million of which less than 5% may be actively synthesising protein at any one time. Certain bacteria are
known which induce tumours in plants by inserting their DNA into the plant's chromosome by means of a vector called a tumour inducing (Ti) plasmid. Attempts are being made to introduce into Ti plasmids desirable genes which can then be transferred into a plant's genome. While intriguing in concept, in practice it is not so simple. First the genes which express themselves in desirable characters in one plant have to be identified, isolated, excised, introduced into the vector, transferred into the plant whose characters are to be enhanced and then persuaded to turn on (express themselves) in that part of the plant where they are needed. In each plant organ: leaf, root, ovary, anther, a different set of specific genes express themselves. Furthermore, many essential plant characters result from the expression of several genes.

It is a popular proposition among journalists that the ability to fix nitrogen displayed by legumes can be conferred upon cereal grains by a transfer of the necessary genes from, say soybeans to wheat. But in legumes at least 17 genes (nif genes) are involved in the process of biosynthesising protein from atmospheric nitrogen. The reduction of atmospheric nitrogen to ammonia whether in a living plant (legume) or a chemical plant (Haber-Bosch process) absorbs a great deal of energy. If the essential nif genes could be transferred, sequenced and induced to express themselves in a cereal grain the energy used could very well show up a significant reduction in yield. Also unless the biochemical pathways in the cereal were appropriately modified the increased nitrogen might deposit itself elsewhere than in the seed or, if in the seed, as non-essential amino acids such as often results when seed endosperm protein is increased by large applications of nitrogen fertilizer.

Greater hope may lie in the genetic manipulation of such nitrogen-fixing bacteria as Rhizobia which infect and cause nodules to form in legume roots where they convert atmospheric nitrogen to ammonia which the plant synthesises into protein. Some success is also reported in the genetic manipulation of free-living nitrogen-fixing soil bacteria by improving their association with the roots of cereal plants.

Most of the work has not yet progressed far beyond the laboratory. To be effective the genetically changed micro-organisms must compete successfully with the natural micro-flora of soils in farmers' fields. Consequently any research to transform Rhizobia genetically in order to improve their N-fixing efficiency needs to be undertaken in close cooperation with plant breeders and soil scientists.

In some quarters it appears to be assumed that plant cell and tissue culture offer alternatives to conventional plant breeding. In the foreseeable future such is unlikely to be the case. Unquestionably they will provide plant breeders with valuable new tools and techniques. Meristem tissue culture permits cassava breeders to generate disease free plants from infected materials. Anther and pollen culture offer significant hope of reducing the breeding cycle to generate improved lines of rapeseed and other economically important crops. The propagation of oil palm from tissue culture has been well publicized.
In all of these successful instances, the tissue culture research was integrated with a conventional plant breeding program to achieve a clearly defined result.

Though many valuable and essential fundamental studies of the biochemical and physiological nature of living cells and the transformations they undergo may be pursued in institutes of molecular biology, applied research in plant cell and tissue culture needs to be integrated with conventional and established plant breeding facilities if the work is to be directed towards an appropriate and useful purpose.
Figure 1

**IN VITRO CULTURE FROM MERISTEM**

1. Plant
2. Remove apical and lateral buds
3. Place in culture
4. Culture initiation
5. Growth in culture
6. Repeated subculture
7. Culture multiplication
8. In vitro rooting (optional for some crops)
9. Return to soil
Figure 2

ADVENTITIOUS SHOOT FORMATION

- Remove explant (e.g., leaf disc or stem section) and place in culture.
- Adventitious shoot formation.
- Callus formation.
- Callus proliferation.
- Rooting.
- Return to soil.

Figure 3

SOMATIC EMBRYOGENESIS

- Remove explant and place in culture.
- Callus formation.
- Liquid suspension culture.
- Or germination.
- Return to soil.
BIOTECHNOLOGY: NEW HORNS FOR AN OLD DILEMMA

JOSEPH H. HULSE, VICE-PRESIDENT, RESEARCH PROGRAMS
INTERNATIONAL DEVELOPMENT RESEARCH CENTRE

PREPARED FOR PRESENTATION TO
FIRST REGIONAL CONFERENCE OF
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BIOTECHNOLOGY: NEW HORNS FOR AN OLD DILEMMA

JOSEPH H. HULSE, VICE-PRESIDENT, RESEARCH PROGRAMS
INTERNATIONAL DEVELOPMENT RESEARCH CENTRE,

Biotechnology - What is it?

"When I use a word it means what I choose it to mean - neither more nor less." Humpty Dumpty's scornful response, when Alice in 'Through the Looking Glass' disputed his misuse of the word "glory", might very well apply to contemporary connotations of "biotechnology".

Technology is defined as: "the practice, description and terminology of those applied sciences which have practical value and/or industrial use". Thus it would seem logical to define "biotechnology" as: "The useful application of all the biological sciences". However, in the 1975 edition of Chambers' Dictionary of Science and Technology, "biotechnology" is defined synonymously with "ergonomics" - the study of work in relation to the environment in which it is performed.

The Canadian Ministry of State for Science and Technology (MOSST) in its publication "Biotechnology in Canada" defines biotechnology as "the exploitation of microorganisms or their components". Then by way of explanation, it adds that biotechnology is "an umbrella term to cover a range of technologies classified into three non-mutually exclusive areas: 1) fermentation technologies; 2) enzyme technologies; and 3) cellular manipulation technologies".

Among others, MOSST's list of biotechnological activities in Canada includes: plant cell tissue culture; embryo culture; somatic hybridization; protoplast fusion; isolation of plant enzymes; biochemicals from animal residues; insect sex pheromones; medical devices (eg. artificial pancreas); biochemistry and metabolism of nucleic acids; extraction and study of plant cell alkaloids; high pressure degradation of lignocellulose; control of food-borne pathogens; cultivation of marine plants; enzymes in macro-algae; immobilization of microbial cells and enzymes (including lactase from whey, fungal amylases and proteases); synthesis and degradation of polysaccharides; synthesis of hormones, antibodies and other pharmaceuticals.

Biotechnology seems thus disposed to embrace virtually all aspects of applied biophysics, applied biochemistry and applied microbiology, together with a fair sprinkling of organic chemistry and chemical engineering added for good measure. Humpty Dumpty would find an etymologically comfortable pew among the biotechnological congregation.
Bury, in his "Ideas of Progress" stated that technology is the basis of modern civilization. In what manner biotechnology in its various interpretations will enhance or distort the lives and environments of future civilizations depends upon the scientific skills, sensibilities and sensitivities that are devoted to its study and application.

Bread and Beer

No biotechnology has exerted a more pervasive and persistent influence on humankind over so long a period as the conversion of carbohydrate to ethanol and carbon dioxide. The distillation of ethyl alcohol was known to the Chinese more than 3,000 years ago and around 1200 BC a Chinese Imperial edict on the subject of alcoholism stated that "spirits are what men will not do without". Bread, beer and wine making were well established in Neolithic times. The popularity of wine in Ancient Greece is revealed by Homer who described it as a gift of the gods. Noah's wine ("Yayin" in Hebrew) is referred to 141 times in the Old Testament. Ecclesiasticus tells us that "From the beginning wine was created to make men joyful, not to make them drunk". Omar Khayyam anticipated the thoughts of thousands when he asked himself "I wonder what the vinters buy one half so precious as the stuff they sell.

Alcohol (al kohl), originally the name given to such fine black powders as antimony sulphide, was applied by the alchemists to mean any very fine powder. During the 16th century it evolved to mean any impalpable or spirituous substance, at which time Paracelsus used "alcohol" synonymously with his distilled "spirit of wine". Our knowledge of the nature of fermentation and ethanol distillation would probably have advanced more rapidly had not several generations of alchemists resorted to the "principle of dispersion" - the deliberate use of maximum words to give minimum information; an artifice employed to obscure what they discovered during the many fermentations and distillations carried out in pursuit of their elixir vitae and lapis philosophorum.

Beer, wine, bread and cheese, all products of microbial fermentation, have doubtless been around as long as people have harvested and eaten cereals, vine fruits and milk. Microorganisms were enhancing and impairing the quality of man's food long before bacteria, yeasts and mould fungi were recognized as such.

Philosophers and historians have long speculated upon which came first, bread or beer. The question is largely meaningless since their origins, raw material substrates, active microorganisms and products of fermentation were and are essentially the same. Both probably had their origins in cereal pastes and porridges which at first adventitiously and later intentionally were inoculated with fermenting organisms.
More than 50 centuries ago the ancient Egyptians and Babylonians converted malted barley and emmer into lightly baked cakes, known as beer bread, which could be eaten directly or stored for later dispersion in water and fermentation into beer. Dried malt and beer cakes were given as wages to serfs in the Egyptian temple administrations. Commercial breweries and bakeries existed in Babylon and Egypt during the second and third millennia. In addition to the attractive stimulation of its alcohol content, beer was less likely than water to induce gastrointestinal disturbances, the Babylonians having unintentionally sterilized the wort by boiling with herbs and spices to extract the flavours.

As is the case with many natural mould cheeses, the microorganisms essential for fermentation were probably carried from one Babylonian beer brew to the next from the cracks in the clay fermenting vessels in which they lodged. At some point a perceptive Babylonian or Egyptian discovered that new brews could be induced to ferment by adding the sedimentary sludge from an earlier exhausted fermentation.

Whiter bread and clearer beer were considered by the Babylonian and Egyptian elite to be of superior quality. Secondary fermentations following sedimentation and the addition of fuller's earth helped to clarify the beer; careful grinding and sieving separated the wheat endosperm as a finer whiter flour from the germ and bran.

Until the mid-19th century bread was leavened by barm, at first from the residual yeast sludge available in breweries, later from a week-long process carried out by the bakers in cool underground cellars. The bakers' barm was grown on a fluid substrate of boiled potato and wheat flour in wooden barrels, the newest batch being inoculated with a scoop from the 7-day old barrel, which was ready to be used to ferment the bread dough. Yeast in the barm fermented slowly so the baker started with a thin liquid slurry adding flour gradually over several hours until the dough was sufficiently viscous to hold its shape, and elastic enough to retain the carbon dioxide as it expanded in the oven.

**Microbiological control**

Following the research of Pasteur and Hansen in the 19th century, panary fermentation was speeded up by selecting fast-acting yeast strains hybridized from sexual spores. In recent years various oxidizing agents and other additives combined with high speed mixing techniques have greatly accelerated fermented bread making processes in European and North American bakeries.

Before Pasteur gave brewers a means of control over unwanted organisms, one can only speculate on how many oceans of beer and wine turned sour. Hops first appeared in Babylonian beer about 200 AD, a period when the northern climate of that region was sufficiently cool and damp for hop cultivation. The first reference to hops in European beer was about 736 AD, beer brewing having moved northwards through what are now the Slavic countries, the Greeks and Romans always having preferred the grape to the grain.
Hop extracts were first reported in 1820 at which time the crude isolate of the yellow phenolic resin lupulon was recognized only as a bitter flavouring agent. It was not until well into this century that Walker at Manchester identified and quantified the bactericidal role of the hop antiseptics humulon and lupulon in inhibiting the growth in beer of acid producing bacilli. It is interesting to speculate if the preference among African rural brewers for pigmented genotypes of sorghum for their kaffir beer is associated with the demonstrable bacteriocidal effect of the polyphenolic procyanidin present in the sorghum pericarp and testa.

The application of microbiological principles aside, one finds little evidence of any fundamental change in milling, baking and brewing technologies over 6,000 years, the major technological developments being devoted to replacing human labour with animal, water and/or mechanical power. Flour milling was the first food process to achieve total continuous flow, the cereal grain being passed by gravity or mechanical conveyor through various break, reduction and fractionation mechanisms until emerging in a variety of fine flours and coarser meals. Though several continuous mixers and extruders have been patented, bread baking remains essentially a batch process up and until the stage at which the dough is divided for proofing and baking.

Mechanization of malting and brewing followed an intriguing pattern leading to Galland's pneumatic malting apparatus in 1885, the Saladin Box with its screws to turn the malt, leading to the more recent Wanderhaufen moving malting couch. Continuous malting and fermentation technologies will expand as brewers make greater use of immobilized enzymes and microbial cells to convert grain starch into what will come to pass for beer.

The chemist has long been an influential actor in the baking and brewing industries, first examining the chemical composition and nutritional composition of the raw materials and the changes they undergo during processing and subsequent storage; later devising chemical modifications that induce process changes economically desirable to the manufacturer and product characteristics pleasing to the consumer. Brewers have used gibberellic acid to stimulate barley germination and an impressive array of natural and synthetic substances, some to clarify the beer, some to stabilize the foam on top.

Bakers have long used oxidizing and reducing agents to modify rheological properties and more recently in combination with high speed mixing, to reduce or eliminate bulk fermentation. They employ surfactants to improve crumb softness and to slow the apparent rate of staling; fatty acids and their derivatives as mould and bacterial inhibitors. More recently fungal amylases and proteases have come to replace or complement the enzymes naturally present in cereal grains and the organisms by which they are fermented in brewing and baking processes.
The Beginnings of Microbiology

Microbes constitute about one-quarter of the world's total biomass. Of the more than 100,000 identified species, no more than a couple of hundred microorganisms are employed to synthesize or modify products useful to mankind. Since however many thousands more engage in the natural recycling of organic substances, it is somewhat misleading to classify actinomycetes, bacteria, moulds and yeasts as useful or useless. It is probably easier to identify those that are pathogenic to living organisms of social or economic importance. Higher orders of terrestrial plants represent 65% of the world's biomass but only 6% are classed as cultivated agricultural crops. Of the 80,000 known edible plants, 90% of the world's food calories are derived from only 12 cultivated plants. Conventional agriculture will therefore continue to provide most of the world's food needs for many decades to come. Nevertheless, the earth's vast unexplored microbiological reservoir offers immense scope for future examination, evaluation and possible exploitation.

Man established his ascendancy over wild animals about 30,000 years ago. He began to control microorganisms when, about a century ago, it was discovered they existed. The known microorganisms employ a wide variety of substrates and metabolic processes to generate the energy and organic material needed for their growth and multiplication. The species of biotechnological value are those whose structural cellular material, natural or modified by-products find useful application.

Natural and modified by-products are classically illustrated by the fermentation of glucose by Saccharomyces cerevisiae (literally: "the sugar fungus which produces beer"). The natural conversion progresses through fructose diphosphate, triose phosphate, phosphoglyceride and pyruvate to carbon dioxide and ethanol. The addition of bisulphite shifts the metabolic pathway and pyruvate is converted to glycerol not to ethanol, a discovery made by Carl Neuberg on which the German explosives industry was heavily dependent during WWI, when 12,000 tonnes of glycerol from fermentation were annually converted to nitroglycerin.

Our present state of empirical and fundamental knowledge has been accumulated over many centuries of observing the consequences of microbial activities. The notion that infections were transmitted from one sufferer to another long predates Pasteur's discoveries in the late 19th century. Primitive people have long held the belief that infectious diseases came as a punishment from the gods and were spread by gaseous evil spirits. More than 2,000 years ago Cicero speculated that fevers were caused by the multiplication of minute animals. We owe to Hippocrates the concept that each disease has its own nature and no one arises without a natural cause. In his environmental studies on "Air, Waters and Places", Hippocrates observed that during the summer months epidemics of dysentery, diarrhoea and Quartan fever occurred most frequently near marshy water. Many of the Ancients held a strong belief in abiogenesis: fleas, bugs and lice originated from filth, fish from mud and, according to Aristotle, eels were formed out of the earth's guts.
In the early 16th century Hieronimus Fracastorius of Verona studied syphilis (the name of a promiscuous character in a contemporary poem) along with several other human and animal diseases. He proposed that these diseases were disseminated through "seminaria" transported by: 1) direct contact (contagion); 2) by air; and 3) by "fomites": porous inanimate objects such as clothing, the seminaria being stored in the pores. He conceived seminaria as chemical substances capable of evaporation and diffusion, and noted that they were extinguished or destroyed by hot or cold remedies, especially by burning.

Van Helmont in the early 17th century first recognized that both alcohol and Gas vinorum (CO₂) were produced during fermentation. It was not until 1766 that Cavendish determined that only "fixed air" (carbon dioxide) was evolved in alcoholic fermentation whereas both fixed and inflammable air (methane) resulted from "putrefactive" fermentation. Van Helmont also believed in the spontaneous generation of animal life from vegetable matter, a proposition disputed by the discoverer of the human circulatory system William Harvey who proclaimed "omnia ex ovo".

Anthony van Leeuwenhoek in 1675 was the first to describe and to draw bacteria which he called "animalcules". Shortly thereafter Spallanzani demonstrated that broth remained free from microorganisms when boiled in sealed containers under pressure. Unfortunately the implications of Spallanzani's work were not appreciated until re-discovered by Pasteur almost one hundred years later.

It was about 1722, while studying alcoholic fermentation, that Lavoisier established that sugar and alcohol consisted of carbon, hydrogen and oxygen and from his observations conceived his principle of the Conservation of Matter, a principle made more precise in 1815 by Gay-Lussack who quantified the conversion of what he named "xymohexose" to ethanol and carbon dioxide.

Pasteur's work, which began about 1857, was devoted to the study of various fermentations including lactic, butyric, acetic and ethanolic. His first attempt to determine the specific action of microorganisms was while investigating a disease of silkworms that threatened the French silk industry. Pasteur identified a microsporidium present on the diseased but absent from the healthy silkworms. The latter he saved by segregation and burning of the infected. Pasteur later gave his attention to the control of the rod-shaped bacilli that convert ethanol to acetic acid in beer and wine. It was he who, in 1871, persuaded the British Whitbreads Brewery to buy a microscope. He also showed the brewers that by heating the wort they could prevent subsequent souring, probably the first recorded demonstration of deliberate "Pasteurization".

Berzelius, von Liebig and Wohler all believed fermentation to be an organic chemical reaction, and that yeast was composed of lifeless organic matter. Using a nutrient liquid medium containing only sugar, inorganic nitrogen and phosphorus, Pasteur grew yeast, thus refuting Liebig's contention that organic matter of animal or vegetable origin was essential to sustain what we now know as microbial fermentation.
One of the most important steps towards modern biotechnology occurred in 1897 when Eduard Buchner macerated yeast cells and showed that the extracted liquid, totally free from living cells, converted sugar to ethanol and CO₂. Buchner laid the foundation for the eventual understanding that each biological conversion depends upon a sequence of biochemical reactions each catalyzed by a special enzyme.

**Biotechnologies ancient and modern**

Almost all traditional systems of food preservation involve the control or utilization of microorganisms. Cooking destroys pathogens and spoilage organisms; less intensive heating reduces active water content to concentrations too low for microbiological proliferation; smoking produces partial dehydration accompanied by the bacteriocidal effects of the phenolic products of pyrolysis. Salt in high concentration dehydrates by osmosis; lower concentrations promote the anaerobic growth of lactic acid bacteria. Lactic, acetic and propionic acids from traditional food fermentations reduce the pH to a level which inhibits the growth of putrefactive organisms.

A comprehensive description of all the foods traditionally preserved by fermentation would be material for a major publication. In addition to the hundreds of cheese types, sour cream, buttermilk and yogurt familiar to North Americans, other lactic milks include kefir and koumiss, popular in Slavic countries, and vilia, a product of Finland. Russian kefir results from milk inoculated with a lactobacillus and a yeast which grow symbiotically to yield an acidified carbonated sour milk. Egyptian kishk and Greek trahana are both made from sour milks boiled with cereal before sun drying. Nigerian ogi, Kenyan ugi, and the South African magou are all products of the lactic fermentation of maize, sorghum or millet.

Korean kimchi, a lactic fermentation of Chinese cabbage, radishes and other vegetables by various organisms including L. mesenteroides and S. faecalis, is typical of pickled vegetables eaten throughout Southeast Asia. For many centuries the Egyptians have made lactic pickles of carrots, turnips, cucumbers, onions, and olives. West African gari results from the fermentation of cassava by species of Geotrichum and Propionobacter. The propionic acid generated stimulates enzymic hydrolysis of the cyanogenic glucoside linamarin present in cassava liberating hydrocyanic acid which steam-distills off.

Indian idli is an acidic steamed bread made by the lactic fermentation of rice and the legume black gram (Phaseolus mungo). Japanese miso, shoyu (soya sauce) and natto are products of soybeans fermented with Aspergillus species; Chinese sufu is a soft cheese-like product from soybean curd fermented with Mucor species. Fish sauces and fish pastes are produced by the autolysis of salted fish in South and Southeast Asia, a region where preservation by autolytic and microbiological pickling and smoking are of long tradition.
For some years a British company has cultured a micro-fungus, the fibrillar mycelium of which is not unlike cooked animal flesh. Modern extrusion technologies that produce meat analogues were anticipated several hundred years ago in Indonesia and other Asian countries where the fibrous mycelia of Rhizopus species grown on soybeans, coconut or groundnut produce, respectively, tempeh kedelek, tempeh dongkrek, or tempah ontjum. Conversion of soybean to tempeh kedelek reduces cooking to one-fifth the time needed for raw soybeans; the crude protein content and digestibility are improved; riboflavin, niacin and vitamin B12 are all significantly increased.

Industrial Biotechnologies

Over interminable centuries traditional medicine applied mould funghi to stimulate the healing of wounds and skin lesions. During the 1870s Tyndall and Roberts described the antagonism displayed by some microorganisms towards others. In 1928 Alexander Fleming's discovery that Penicillium notatum destroyed laboratory cultures of Staphylococcus aureus led to the discovery and eventual isolation by Florey of penicillin.

Following Waksman's antibiotics from actinomycetes came a proliferation of fermentation processes and products. It is estimated that about 300 new antibiotics are discovered every year most from species of actinomycetes mainly of the Streptomyces genus.

The useful natural and modified products of industrial microbial fermentation are many and varied; they include familiar substances originally made by chemical synthesis and a growing number of novel and unusual compounds. The spectrum includes alcohols, organic acids, aldehydes, ketones, amino acids, vitamins, enzymes, flavor enhancing nucleotides, carotenoids, and a rapidly expanding range of pharmaceuticals and bioinsecticides. Among the unusual is the carotenoid astaxanthin derived from the yeast Phaffia rhodozyme which converts the natural white flesh of cultured salmon and trout to a more desirable pink colour. Industrial products of fermentation include more than 90 antibiotics, over 25 enzymes, about a dozen organic acids, close to 20 amino acids and a dozen or more vitamins and nucleotides. Industrial production of enzymes has grown from roughly $5 M per annum in 1960 to several hundred times that value today. One of the most rapid growth demands has been for bacterial proteases by manufacturers of laundry detergents. The products of microbial fermentations are estimated to earn about $15 billion annually for Japanese industries.

More than 120 companies in the wealthier countries are engaged in fermentation industries. Lactic acid has been manufactured in the United States for more than 100 years. Glucose isomerase which converts glucose into fructose is satisfying a much more recent industrial demand. The manufacture of fructose in the United States now exceeds 1.5 M tonnes annually. Bacterial $\alpha$-amylase followed by a fungal glucoamylase convert a liquid starch slurry to crude glucose solution, the composition and pH of which are adjusted before continuous conversion to fructose by immobilized glucose isomerase. Efforts
to increase fructose yields include studies of isomerases from different microorganisms, various techniques of enzyme immobilization and reactor design, followed by fractionation of the saccharide mixtures generated.

Though Buchner's classical experiment was often repeated, one of the earliest reports of isolated enzyme immobilization - laboratory experiments with invertase - appeared in the Journal of the American Chemical Society in 1916. One of the first industrial processes used immobilized amino acylase to separate the acyl derivatives of racemic mixtures of amino acids customarily produced by chemical synthesis. Synthesis by microbial enzymes will produce the desirable L-amino acids rather than racemic mixtures.

In a very recently reported development haemoglobin immobilized onto a spongy polymer - a Hemosponge - absorbs oxygen from deep sea water. The oxygen can later be released by a weak electric charge to be breathed by submariners or to oxidize fuels in submarine engines.

Techniques of immobilization and reaction are diversifying more rapidly than industries can adopt them. Enzyme immobilization includes absorption, micro-encapsulation, gel entrapment and covalent bonding with a wide and growing range of solid and porous-organic and inorganic materials. Continuous reactors include packed columns, fluidized and trickle beds, membranes and spun fibres. While some of the techniques have been borrowed from industrial chemistry, the lability, reactivity and environmental sensitivity of isolated enzymes present unique challenges both to chemical engineers and biochemists.

Though chemical additives can change metabolic pathways and products of fermentation, genetic modification and mutation offer greater opportunities by which to increase the output of normal metabolites or to generate products abnormal to natural metabolic processes. Genetic mutations were first induced by x-rays, subsequently by other forms of high energy radiation and chemical mutagens. Recombinant DNA and molecular cloning techniques derived over the past decade provide a more potent means of genetic programming in microorganisms. Protoplast fusion and plasmid transfer offer intriguing possibilities for the future production of antibiotics, amino acids and important chemical macro-molecules that are difficult and expensive to synthesize by conventional chemistry.

Responding to the wealthier nations' concern for their health and longevity, biotechnological industries now devote significant resources to improving the manufacture of antibiotics and amino acids. In the U.S.A. the annual market value of antibiotics and vitamins respectively exceed $900 M and $130 M. Many cycles of mutation and selection have generated mutants of Penicillium chrysogenum and Streptomyces aureofaciens with significantly higher antibiotic yield capabilities than the original wild strains. The Japanese have successfully selected auxotrophs of Corynebacterium glutamicum which produce high yields of threonine at the expense of the other two amino acid metabolic products. Higher threonine yields are reported from C. glutamicum modified by gene splicing.
Fuel, Feed and Food

About a decade ago, when a barrel of oil ceased to cost the same as a bushel of wheat, North Americans came under intense economic pressure to discover new sources of combustible fuel. Because, scientifically and industrially it was best known, ethanol from fermented carbohydrate appeared as the logical alternative to high priced gasoline. However, in publicizing the potential merits of gasohol, and indeed of many other potential biotechnologies, the popular media tend to muddle scientific fact with science fiction. Dr. Gregory at the University of Guelph has calculated that to substitute gasohol (10% ethanol) for all the world's automobile fuel would require as much land be given to carbohydrate crops as is now used for all food crops. Total replacement of gasoline by ethanol would absorb 10 times the land now devoted to cultivated agriculture.

Considering that the annual global loss of agricultural land is about 15M ha, 8M ha of which is destroyed by urban and industrial development (3M ha in developed countries), ethanol from cultivated carbohydrate crops does not appear very attractive either internationally or in Canada where, according to the Department of the Environment, we have lost more than 1.5M ha of farmland over the last 20 years. Lignocellulose from wood and grain straw appears as a more attractively abundant raw material than carbohydrate for microbiol conversion to fuel provided that as much critical attention is devoted to potential market demand and production economics as to processing technologies. Several high pressure steam technologies to convert lignocellulose to cellulose, hemicellulose and lignin have been described but these have yet to be proved economic. More extensive collection, screening and selection of mesophilic lignocellulolytic funghi offer opportunities as yet unexplored particularly for tropical countries. Several research groups are collecting and screening potentially useful organisms such as wood rot funghi.

More extensive use of biogas derived from human, animal and other organic waste is constrained not only by aesthetic but logistical difficulties. In the short term the best opportunities for biogas would appear to exist at large animal production units and municipal garbage collection sites, particularly if the fuel gas can be consumed close to the point of generation.

More than a decade ago conventional nutritional wisdom proclaimed pervasive protein deficiencies throughout many developing countries. More recently protein deficiency is said to be less serious than was originally thought, mainly because nutritional expertise has revised and reduced the earlier recommended levels of daily intake. Nevertheless, scientific and in less degree industrial interest in producing microbial protein for human food and animal feed is still evident. The yeasts S. cerevisiae and Candida utilis were grown for human food in Germany during WWI and II. Until petroleum prices began to soar, most of the Seven Sisters and their competitors were exploring the conversion of low and high molecular weight hydrocarbons to microbial protein. Now only two companies in the western world, Hoechst AG in the Federal Republic of Germany and ICI in the United Kingdom appear active in the industrial conversion of methane (first oxidized to methanol) into
microbial protein using strains of Methylophilus methylotrophus. In the USSR, where there seems to be less concern about true cost and safety, at least a dozen factories are said to be manufacturing microbial protein from hydrocarbons. The attraction of the microbial cell, yet to be extensively exploited, lies in its high surface to volume ratio, which permits a rapid transport of nutrients to support a high metabolic rate. Thus the rate of production of protein in yeast is several orders of magnitude greater than in soybean.

**Nitrogen Fixation**

Rapid price escalation and uncertain availability of chemical fertilizers in many developing countries has excited interest in and in some cases unreasonable expectations from the reduction of atmospheric nitrogen by soil microorganisms. More than 25 genera of free living soil organisms which fix nitrogen for their own needs have been described. Much of the nitrogen fixed in the rhizosphere by free living organisms remains unavailable until the microbial cells decompose. In common with chemical fertilizers, the fixed nitrogen is also subject to loss by leaching.

Probably the most widespread of the free-living nitrogen fixers are the blue-green algae, some species being adapted to terrestrial others to aquatic conditions. Some survive in the Antarctic at near freezing temperatures, others above 60°C in hot springs. The association between Anabaena azolla and the aquatic fern Azolla pinnata in tropical aquatic environments reportedly produces up to 150 kg N/ha/yr, the azolla being used to fertilize rice paddies or as forage for ducks and pigs.

The associative symbiotic relation between Digitaria decumbens and Azospirillum lipoferum has excited the hope that the more efficient C-4 photosynthetic cycle in tropical grasses could satisfy the Azotobacter's high energy demand estimated to be equivalent to about 50 kg of sucrose to reduce one kg of nitrogen. To this end more needs to be learned about the physiology of the Azotobacter and the biochemical relations between identifiable species and strains in association with defined tropical grass genotypes.

In an elegant summary of the present state of knowledge that underlines the errors inherent in extrapolating potential nitrogen fixation per ha/yr from a few acetylene reduction determinations, Beringer suggests how ¹⁵N compounds can be used to assess nitrogen fixation in selected crops over a growing season. Beringer also summarizes the many constraints to the effective transfer and expression of nif genes into cereal grains, an objective unlikely to be achieved for many years but one much touted by popular science writers as an imminent benefit to world agricultural production. Beringer lays much stress on the energy cost of nitrogen fixation pointing out that photosynthate used to energize nitrogen fixation could well be expressed as a significant yield loss. He argues that in tropical developing countries where crop yields are low and not limited by water, excess photosynthetic capacity available may be sufficient to fix nitrogen without cost in terms of crop yield. Beringer concludes that "for the
foreseeable future research on nitrogen fixing cereals and other crops will be of only marginal benefit to the developed countries though it should be of great value to the developing world."

Of particular interest to foresters are the actinomycetes and other non-specific endophytes which stimulate nodulation in non-leguminous tree species where instances of nitrogen fixation in excess of 350 kg/ha/yr have been reported. The symbiotic relation between Frankia and Casuarina species is of particular interest for semi-arid and saline soils, the techniques of culturing Frankia now offering hope of large-scale inoculant production.

Biotechnology in Canada

Recent publications by the Ministry of Science and Technology (Biotechnology in Canada, June 1980), the Science Council of Canada (Biotechnology in Canada - Promises and Concerns, September 1980), and the Canadian Agricultural Research Council (Biotechnology Research and Development for Canada's Agriculture and Food System, April 1983) present catalogues of and commentaries on biotechnological research and development in Canada.

In the agricultural sector greatest effort is apparent in crop improvement research through embryo culture, sexual and somatic hybridization, together with growing interest but so far modest activity in gene transfers between unrelated organisms. In the realm of plant protection it is sometimes difficult to distinguish between conventional plant pathology (i.e. breeding and selecting for resistance; etiology; biochemical and physiological symptoms) and the application of novel biotechnological methods. Among the latter, greatest activity seems to be concentrated upon viruses and viroids including production of hybridoma lines, synthesizing monoclonal antibodies against particular viruses or viral strains, and the selection of monoclonal species suitable for specific analytical and/or diagnostic purposes.

There appears to be a growing interest in identifying and intensifying the natural enemies of important pests and pathogens, and the tissue culture of natural enemies on the susceptible organic cells of host pests. A particular example of the latter is the culture of microsporidia on the abdominal cells of grasshoppers, a technique now being studied as a potential means of controlling locusts in India.

Research on nitrogen fixation seems spread unevenly over a wide spectrum that embraces several plant-microorganism relations and systems. A more comprehensive coordination and sharper focus though desirable, seems somewhat constrained by the difficulty of determining precisely the contribution made by nitrogen fixation to agricultural production.

Improvements to animal production and health are being sought through recombinant DNA technology in the production of vaccines; monoclonal antibodies to replace serum-derived antibodies in the diagnosis and monitoring of animal diseases; genetic manipulation of embryos; sexing and cryopreservation of embryos; and sex selection by semen fractionation.
Among fermentation technologies carbohydrates and to a lesser extent lignocellulose appear as the dominant substrates. Some interest is being shown in methane generation though the extent of industrial involvement is not clear. In spite of sizeable imports relatively little biotechnological activity seems to be devoted to enzyme, vitamin, amino acid or nucleotide production. Investment by Canadian pharmaceutical companies in research and development (less than 5% of sales) is much lower than the average R and D expenditures among the world's leading pharmaceutical companies (close to 12% of sales).

A criticism common to the reports cited above and others on the same subject is that Canada's investment of human, material and financial resources is inadequate if we hope to carve a significant future niche in industrial biotechnologies. An inadequate human resource is by no means confined to biotechnology. In 1973-74 Canadian universities graduated roughly 760 people with doctoral theses in the physical and applied sciences. By 1978-79, the number of doctoral graduates in these disciplines had dropped below 450. A recent report indicates that many more Canadian agricultural research scientists will retire during the next five years than will be replaced by new doctoral graduates. Though we need about 150 new PhDs annually, our eight faculties of agriculture are graduating less than 70.

These melancholy facts foretell intensive competition between conventional agriculture and biotechnology for the minds and bodies of young Canadians disposed towards and capable of research of the high quality to which we have become accustomed. Clearly there are some exceedingly difficult scientific and technological decisions to be made in determining our scientific priorities.

The Future: Pleasant Promise or Ominous Risk

The Pragmatist American philosopher John Dewey wrote that "To the person fully alive, the future is not ominous but a promise; it surrounds the present like a halo". Eighteen centuries earlier the Stoic philosopher Epictetus advised: "If you wish to live a life free from sorrow, think of what is going to happen as if it had already happened."

In an important paper to the Science Council of Canada in 1980 David Suzuki stated: "Because implications of the new (recombinant DNA) technology are enormous I sound a cautionary note based on historical precedent...There will be unexpected and detrimental effects of the new DNA technologies. I say that without direct documentation but confident from history's lessons that there are no problem-free technologies and no fool-proof systems. Remember, it is fallible human beings...who designed and used the systems in the first place...Precautions often slow down research and biotechnology in an area characterized by incredible speed and competitiveness. If you are in competition and have to accomplish something in a short time, then paying attention to what you consider unimportant precautions is not going to be a high priority. I am not convinced that Canadians have come to grips with the difficult issue of how to enforce safety regulations."

Silicon Valley and its inhabitants have witnessed the rapid rise and fall of many enterprises in the high technology communications and computer industries. But they deal with inanimate, inorganic materials whose
interactions are more predictable and controllable than biologicals. Even our oldest established biotechnologies, brewing and baking, rely as much upon empiricism as upon sound science. Consequently, not all of what is proposed by the more enthusiastic advocates of biotechnology is predictable in terms of economic viability, social acceptability and most important, human safety. Many pundits appear more in harmony with Dewey than with Epictetus.

The synthesis of substances for pharmacological investigation by genetic programming of microorganisms seems almost limitless. In the technologically advanced nations antibiotics, antiviral agents and other therapeutics undergo extensive laboratory screening before being administered to sick people under medical supervision. Most developing countries are less favourably possessed of the laboratory facilities and professional competence necessary for comprehensive biological screening.

The genetic programming of unfamiliar microorganisms to convert various waste materials into protein for food or feed, while scientifically intriguing, gives cause for considerable concern. It is doubtful if any reliable method exists by which to determine the absolute safety for continued consumption of totally novel foods derived from fermentations of genetically manipulated microorganisms. Microbial fermentations do not produce single pure substances. What appear as high protein products from fermentations by genetically restructured organisms might well contain substances unknown, unanticipated and undesirable for long term ingestion.

Without doubt biotechnologies, wisely selected and applied will prove beneficial to agriculture, medicine and industry in both developed and less developed countries. It is to the latter that biotechnology presents greatest potential promise but where novel processes and products must be approached with greatest caution. If Canada's biotechnological capability is weak, the scientific, institutional and human resources of most developing countries are considerably weaker. For example, in 1978 in all the countries of Latin America and the Caribbean graduates in natural sciences constituted only 2.6% of the total. Social sciences, humanities and law represented 65%, agriculture 4.4%.

Under no circumstances should the poor developing countries be regarded as the testing grounds and their people as the world's guinea pigs for the novel products of biotechnology. A proposal in a recent Canadian document that Canada produce microbial protein for export to developing nations is unworthy of serious consideration. If we won't eat it, why should they? Furthermore, North American and European manufacturers should be prevented from exporting to developing countries pharmaceuticals of a nature or quality unacceptable to or inadequately tested in their own countries.

The realization of the many potential benefits of biotechnology demands a very high order of scientific excellence: excellence in conception, excellence in investigation, excellence in application.
But in what should we seek to excel? Given the biotechnical smorgasbord before us, which of the array of alternatives do we pursue? Bearing in mind the decline in research graduates in the biological sciences how do we choose our priorities and resolve the inevitable competition for scarce talent between the new biotechnologies and the established conventional agricultural technologies upon which so great a proportion of our national economy still depends? This is the dilemma which faces us and many other nations: how best to allocate our limited scientific and industrial resources between strengthening what is established and pursuing what is novel.

Who Will Choose the Priorities

Most difficult to decide is who is competent to make wise biotechnological choices. For the most part, the task is quite beyond the ken and capability of politicians. Politicians rarely display an informed awareness of how science functions or what is needed to develop a national superiority in pure or applied science. The distribution of research funds according to the dictates of political whimsy or expediency encourages mediocrity and frustrates the pursuit of excellence.

Contemporary trends and patterns of ownership and management in North America do not inspire confidence in industry's ability to make wise biotechnological choices. Over the past three decades the proportion of senior industrial executives with professional degrees in science has declined markedly in favour of presidents with legal and financial qualifications. Corporate industrial ownership displays a disturbing disposition towards monopolies in which enterprises widely divergent in technological and marketing activities are controlled by a single financial holding corporation. The corporate philosophy of these monopolies seems generally more disposed to the manipulation of a stock market portfolio than to the creative management of applied science.

Realization of the opportunities from biotechnology requires an educated imagination, courage and patience; attitudes often inconsistent with the stock portfolio pursuit of relatively short term gain; the primary preoccupation being with the bottom line of the next quarter's profit and loss statement. The myopic outlook of the stock investment mentality is evidenced first by the eccentric fashion in which the share prices of the genetic engineering companies have yoyoed up and down in the U.S. stock markets; second, by the rapid retrenchment in industrial research investment as the recent recession developed. Closure of a research division can boost a company's operating balance in a manner immensely pleasing to the bottom line watchers.

To make reliable choices, to plan and direct the research and development to elaborate sound and economic biotechnologies, and to deliver the products to a receptive market is no small undertaking. The rate at which new knowledge and new techniques are being generated is almost overwhelming. Furthermore, the field suffers from its high publicity profile, with the attendant danger that results will appear in the popular press before being
subjected to peer scrutiny. Nevertheless, it is a field with exciting possibilities. A field in which Canadian science can excel. But it will need a new breed of scientific managers to direct it.

The scientists who chart the course towards biotechnological fulfillment need a breadth and depth of vision and comprehension not always evident in people whose minds have been trained and devoted to a sharply focussed specialization. Unquestionably, highly specialized competence is essential to the realization of the scientific opportunities that lie ahead. But the specialists need to be guided by managers of research and development: people who are as cognizant of the potentialities and perils of the market place as of the scientific principles and practices by which biotechnologies are conditioned.

Biotechnology combines the elements of both the natural and the social sciences. Reliable biotechnological forecasting calls for the ability to define, analyze and stimulate potential markets; combined with an exceptional breadth of scientific understanding. Most important, it calls for a social conscience sufficiently refined to ensure that biotechnology will seek first and foremost safely to satisfy the needs of humanity.

This paper began with Humpty Dumpty - who sat on a wall and had a great fall. If the new biotechnologies are to reach the heights of greatest expectation and to be spared Humpty Dumpty's fate, they will need to be planned, programmed and directed by a special breed of scientific managers: people guided by Epictetus' dictum; men and women able to examine each biotechnology in its total scientific, technical and social environment. There is no room for enthusiastic amateurs or those of limited comprehension and perspective.

If we in Canada so choose, we can identify and develop the necessary management skills. In so doing we will bring benefit not only to ourselves, but set an example worthy of being followed by many other nations.
BIOTECHNOLOGIES: RESPONSIBILITIES, PRIORITIES AND CONSTRAINTS

JOSEPH H. HULSE, VICE-PRESIDENT RESEARCH PROGRAMS
INTERNATIONAL DEVELOPMENT RESEARCH CENTRE

Presented to: Inter-Center Seminar on IARCs and Biotechnology
27 April 1984, IRRI, Los Banos, Philippines
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BIOTECHNOLOGY: BANE OR BOUNTY

"I look upon all the world as my parish" wrote a famous 18th century evangelist. One wonders how many of his converts became biotechnologists.

In an earlier paper I reviewed briefly several definitions of "biotechnology" and suggested that the word seems disposed to embrace all aspects of applied biophysics, biochemistry and microbiology, together with a fair sprinkling of organic chemistry and chemical engineering added for good measure.

In spite of the short history of its more novel attributes, papers to this meeting show evidence of some quite remarkable progress, much of potential benefit to agricultural sciences.

Biotechnology has proved a doughty competitor with space exploration and advanced electronics in attracting the attention of the popular press, whose judgement of its potential impact ranges between unbounded optimism and deep concern for the future of the human race. The pessimists, who appear to be in the minority, see the biochemical manipulation of life processes as the ultimate revelation of Mary Shelley's Dr. Frankenstein. They seem to share Mrs. Shelley's view that those who unlock the secret of heredity and learn how to create life artificially may not cherish their synthetic progeny with the tender loving care and caution they deserve.

At the other extreme are those who predict boundless benefits, any attendant risks being purely conjectural and improbable.

The following are recent quotations from two different Asian newspapers:

"Biotechnology has been identified as the growth industry of the future because it holds the promise of a single technology that could meet all man's needs."

While welcoming the reporter's enthusiasm, his concept of a single technology comes close to a reductio ad absurdum.

The views expressed in this paper are those of the author and not necessarily of the International Development Research Centre.
The second journalist writes:

"The use of genetic engineering in the West to mass produce crops may render (Asian) agricultural produce - including rice - uncompetitive in the international market. Because of recent advances in genetic manipulation technology, the United States is able to produce more, better and cheaper crops in less time than Asian countries can produce them with ordinary agricultural techniques."

On the other hand, a senior American professor of biology has stated that:

"Science has become too potent and it is no longer enough to wave the flag of Galileo (in its defence). People seriously wonder if, through our cleverness, we shall not blunder into worse dilemmas than we seek to solve."

The professor is concerned that genetic engineering might inadvertently provide deadly weapons to enable the anarchists and the tyrannical in our society to imprison our liberty and threaten our existence.

An eminent Canadian geneticist and science educator has sounded yet another warning:

"Because implications of the new (recombinant DNA) technology are enormous, I sound a cautionary note based on historical precedent...there will be unexpected and detrimental effects of the new DNA technologies. I say that without direct documentation but confident from history's lessons that there are no problem-free technologies and no fool-proof systems. Remember, it is fallible human beings...who designed and used the systems in the first place...Precautions often slow down research and biotechnology in an area characterized by incredible speed and competitiveness. If you are in competition and have to accomplish something in a short time, then paying attention to what you consider unimportant precautions is not going to be a high priority. I am not convinced that Canadians have come to grips with the difficult issue of how to enforce safety regulations."

Notwithstanding its prophets of doom, biotechnology has enjoyed a relatively favorable press, particularly when compared with nuclear physics. Perhaps we should remember that 20 years ago nuclear fission was being heralded as the ultimate solution to mankind's increasing demands for energy in addition to benefits offered by induced mutation to gamma gardens; to the control of cancer; and to the elimination of waste and spoilage from all post-harvest food systems through radiation sterilization.
Not all of these promised benefits are readily evident in today's world.

While welcoming the generally favourable publicity given to biotechnology both by reputable science writers and the more popular press, greater attention seems to be devoted to scientific principles and techniques than to the overall comprehensive management of biotechnological research and development: in particular to the resources needed to pursue to the point of demonstrable human benefit all the many options presented in the biotechnological smorgasbord.

THE EXPANDING AGENDA

The component platters offered in the biotechnological smorgasbord increase with every related conference, symposium and statement of national science policy. The following are lists of subjects recommended for research and development in developing countries, the first from an international agency, the second from a major international conference.

The international agency recommends research be pursued on:

1. Genetically manipulated bacteria to produce energy and fertilizer from biomass;
2. Development of human and animal vaccines;
3. Improvement of traditional and novel fermentation technologies;
4. Improved agricultural plant and animal products using genetic engineering;
5. Drugs and pharmaceuticals for all tropical diseases;
6. Tertiary oil recovery from petroleum wells.

The above presents a disarmingly simple list which obscures a vast, complex and formidable hidden agenda.

The international working group presented an equally daunting list, its suggested priorities being:

A. Biological conversion of agricultural and industrial wastes and by-products:

The conference listed over 30 such by-products but since these included such general descriptions as straw, oilseed cakes, fruit peelings, cannery effluent, slaughterhouse waste, lumber and paper mill waste, and oil refinery hydrocarbon waste, a more specific list would likely be longer by several orders of magnitude.
In pursuit of the conversion of wastes and by-products the working group recommended the following:

(i) Systematic identification and classification of all wastes and by-products;

(ii) Analysis of constituents;

(iii) Identification of the most suitable microorganisms and fermentation processes to achieve economic conversion.

Given the immense natural variation and the rapid changes in chemical composition which biological materials undergo post-harvest and post-mortem in the tropics, together with the thousands of identified and as yet unidentified bacteria, moulds and yeasts that might be examined, this recommendation alone if taken to its logical conclusion could occupy several generations of the world's microbiologists and chemists.

B. The second priority was given to research to gain greater scientific understanding and control of traditional and novel food fermentations.

This clearly deserves serious consideration since one would be dealing with foods that are already accepted, that have been consumed over many centuries and therefore present less potential obstacles to commercial marketing and concerns for human safety. Indeed, the products of panary, alcoholic and lactic fermentations have been consumed for many thousands of years. In spite of this immense history of human experience and the very sizeable research investment over the last 100 years into the technologies of breadmaking, alcoholic beverages and cheese making, these industries are still guided as much by the empiricism of the educated thumb and experienced taste apparatus, as by sound scientific knowledge.

C. The next recommended priority was for embryo, tissue and cell culture research to produce secondary metabolites of commercial value. The international working group listed 54 classes ranging alphabetically from alkaloids to vitamins.

Once again, when each of these general classes is separated into individual substances, the list increases many fold. Little mention was made of the extensive manufacturing and marketing experience, resources and facilities needed to convert laboratory research results into viable industrial technologies.

Furthermore, unlike those primary proteins which are the immediate products of a single gene, are not the antibiotics and other secondary metabolites polygenically controlled? While
recognizing recent progress made by the fusion of cells from different *Streptomyces* species, most of the increases in antibiotic yields have resulted from conventional selection among random mutants combined with improved fermentor design and operation.

D. The fourth priority was given to the microbiological generation of industrial chemicals. Specifically mentioned were alcohols, aldehydes, ketones, glucose and fructose from waste cellulose and lignocellulose. There is logic in such a recommendation in that the three essential components of woody plants: cellulose, xylan (a hemicellulose), and lignin, are nature's three most abundant polymers. The international working group specifically recommended that cellulases from *Trichoderma*, *Xanthomonas* and *Cellulomonas* spp be immobilized to permit continuous conversion of cellulose and hemicellulose to fermentable saccharides.

As is well known, the lignocellulosic complex consists of microfibrillar bundles of crystalline cellulose which provides the skeleton of the fibre surrounded by a matrix of cross-linked xylan and lignin which holds the structure together. It is the lignin/xylan compound which prevents penetration by acids, microorganisms or enzymes.

The first problem is to separate the crystalline cellulose from the amorphous hemicellulose and the lignin. Significant attention has been given to wood rot fungi and other lignocellulytic organisms but to my knowledge, no microbiological fermentation process starting with lignocellulose as it comes from the woody plant has been successfully elaborated to any commercial scale. In fact, some of the microorganisms described appear to take the cellulose all the way to carbon dioxide which is not particularly useful either as feed or fuel.

A promising means of separation which has been experimentally applied to various species of wood, straw stalks and bagasse relies upon explosive decompression to separate the cellulose from the hemicellulose and lignin. Steam at high pressure is injected into the wood chips or chopped straw. As the temperature is raised to about 234°C, the constituents undergo morphological change: first the lignin, then the hemicellulose and finally the cellulose soften and the chemical cross links are so weakened that when the pressure is suddenly released the liquified lignin and hemicellulose are sufficiently disassociated from the cellulose fibres that the three can be separated, the lignin in ethanol or methanol, the xylan by mild sodium hydroxide.

It is claimed that if the process is accurately controlled, the three fractions emerge relatively pure and chemically reactive. The lignin is thermoplastic and can be chemically
converted to a variety of products or used without further degradation; the two carbohydrate fractions must be separated and hydrolyzed by enzymes, microorganisms or mineral acids.

While this explosive decompression technology has been known for some time, it does not appear to have been widely exploited commercially and there is probably still much to be learned about the production economics.

Furthermore, the proposed conversion of cellulose by immobilized cellulase will also call for a significant research program. As mentioned in my earlier paper, in North America much publicity has been given to the conversion of dextrose to the much sweeter fructose by immobilized glucose isomerase. Fructose is now widely preferred over sucrose in soft drinks and many confectionery products. It should be mentioned however that in spite of the attractiveness of fructose to the soft drink and confectionery industries it has taken nearly 10 years to fully establish the industrial technology and to increase production from 0.3 to 3.0 million tonnes per year in the United States. Research is still in progress exploring bacterial rather than fungal sources of isomerase and in improving the carriers upon which the enzymes are immobilized. The various techniques of immobilization include: micro-encapsulation, gel entrapment, covalent bonding to various porous and solid surface polymers, in packed columns, fluidized and trickle beds. Other techniques are still coming.

Consequently, the commercially acceptable conversion of lignocellulose to useful industrial chemicals will necessitate a sizeable investment in applied research and process development, together with an equally comprehensive investigation of the production economics and marketing potential, aspects which tend to be overlooked in much of the biotechnological literature.

E. The working group's fifth priority envisages an impressive range of pharmaceuticals to be generated and eventually manufactured by genetically manipulated microorganisms. In addition to such familiar biochemicals as human insulin and the various interferons, the working group proposed research on hormones for tissue generation, pain and appetite suppressants and ovulation stimulants together with a long list of enzymes and vaccines for the treatment and prevention of a great many human and animal diseases.

One commentator on the working group's recommendations suggested that greater attention be given to vaccines for animals than for humans since the former offer greater potential profit. It was argued that if the vaccines are successful, human beings require relatively few and infrequent doses to acquire immunization. On the other hand, farm animals are slaughtered and
therefore every new animal needs individual immunization. It was also mentioned that the safety requirements for animal vaccines are much less exacting and therefore the cost of bringing them to market is lower than for human vaccines.

THE DRUG DILEMMA

A particular word about pharmaceuticals may be in order. As populations get older and richer, their concern with health and fighting off disease becomes increasingly evident. In spite of George Bernard Shaw's dictum, most of us would probably like to live forever.

In 1980, the estimated value of pharmaceuticals throughout the world was about $75 billion and one agency has forecast an increase to more than $250 billion by the end of the century, of which the LDCs' demand will be about one-third. The U.S. enjoys about one-tenth of the world market ($7.5 billion) of which close to 20% are drugs derived wholly or partially from microorganisms. Antibiotics are the largest class of microbiologically derived drugs, representing roughly 10% of the total world pharmaceutical market.

Over 25 years, the fermentor yields of penicillin have increased from a few milligrams to over 20 grams per litre. The improvements result in part from genetic selection among random mutants combined with significant improvements in fermentor design and operation. It is probable, in the short term, that improvements in antibiotic production will result more from established microbiological methods than from genetic engineering. Unlike those primary proteins which are the immediate products of a single gene, antibiotics are secondary metabolites and thus are the ultimate products of actions of anywhere up to 30 genes. However, the progress made in generating new and modified antibiotics through protoplast fusion of different species of Streptomyces has already been mentioned.

Though the research interest and investment is considerable, relatively few drugs of any kind, produced by the genetic engineering of microorganisms, have yet reached the commercial market on any scale. Developing countries clearly need to develop greater self reliance in the production and distribution of essential drugs. Nevertheless, the constraints for most of them are very great indeed.

The International Organization of Consumers Unions has repeatedly drawn attention to the selling of drugs in developing countries that are restricted or banned in the industrialized countries in which they originate. A recent report illustrates how anabolic steroids and other dangerous drugs can be bought without medical prescription in many developing countries. The report states:
"Many developing countries have few or poor regulations covering what information must be provided about drugs offered for sale, few quality control checks, health systems too burdened to identify which drugs are really needed, and medical authorities with such limited access to information that they rely heavily on product advertisements. Worse, prescription drugs can be bought over the counter in many parts of the third world."

Industrialized countries such as Canada have established extremely rigorous testing protocols by which to establish the suitability of new drugs for use by humans. Canada's Department of Health and Welfare protocol starts by stating: "All drugs produce toxic effects...The ultimate objective is to evaluate the probability that the drug will not produce significant damage under specific conditions of use." The testing protocols include among a long list of extensive evaluations of primary and secondary pharmacological actions: Determination of acute and long term toxicity; carcinogenicity, and mutagenicity; possible effects upon reproduction and teratology; and potential genetic damage.

It is not surprising that the established pharmaceutical companies estimate that clinical trials of each new drug cost $20 million or more. The larger drug companies with established research and manufacturing facilities provide for 8 to 10 years and up to $100 million in investment to bring a new drug to the market. The largest pharmaceutical companies invest more than 12% of their sales income on research and employ large, highly trained sales forces to market their drugs.

The demonstrated possibilities of employing genetically manipulated microorganisms to produce insulin, growth and reproductive hormones normally extracted from the organs of dead animals, have stimulated the rapid rise of a new type of commercial technological enterprise. Several of these companies metamorphosed from university laboratories that had developed a high degree of competence in the production of bacteria instructed by genetic manipulation to make specific proteins to order.

Successful biotechnology companies such as Biogen, Cetus and Genentech have succeeded largely by collaborative arrangements in which larger established companies assume the responsibility for further development and marketing of the biotechnologically derived drugs. Several of the biotechnological research enterprises are now seeking to expand their scope of operations into manufacturing and marketing. In addition to the larger profits realized from the manufacture and sale of drugs, the biotechnological research enterprises have come to recognize the difficulties in being remote from the market place. Success in the pharmaceutical industry
requires more than exceptional research competence. It calls for skill and experience in expanding from a test tube or a small batch fermentor to a full-scale manufacturing technology. It needs a constant direct feedback from the production factory floor; from the market place; from the medical profession engaged in clinical trials; and from those who prescribe and distribute its products.

To metamorphose from a research facility to an integrated production and marketing enterprise requires a very large injection of risk capital in addition to a sizeable increase in competent experienced staff. It seems possible that changes in U.S. tax laws may enable the biotechnological research companies to acquire some of their risk capital from wealthy private individuals who can write off their investments as tax losses.

FROM THE LABORATORY TO THE MARKET PLACE

But what useful lessons does this experience offer to those developing countries who do not have access to the human, material, manufacturing, marketing experience and facilities needed? Many government owned laboratories find difficulty in developing products and technological processes that are adaptable and profitably exploitable by commercial industries whether the latter are privately or parastatally owned. Without an integrated marketing and market research facility, research laboratories cannot easily develop products and processes that are commercially viable or which can be exploited to serve a human need or a user demand.

Illustrative of the investment in time and facilities needed is the experience of a Canadian public company which, for about 60 years, has manufactured and sold insulin, together with a long and expanding list of vaccines and biologicals for prophylactic and therapeutic use.

About four years ago the gene for human insulin was transferred to and expressed itself in a bacterium. The final broth from this bacterial fermentation contains a pro-insulin precursor which, after isolation, undergoes a series of 10-15 chemical modifications before the human insulin analogue reaches the desired degree of potency and purity. It has taken more than four years from the time the insulin gene expressed itself in the bacterium to reach the present advanced laboratory scale of development. It will probably be another two to three years before full commercial production is attained. The work has been carried out by a staff of over 15 scientists and medical professionals, many with more than 15 years post-doctoral experience. The investment necessary has been close to $10 million. A sizeable further investment will be needed before scale-up to full production is accomplished.
As the Director stated, the research is not the most difficult component. The greatest investment and risk lies in the scaling-up to production level; maintenance of fermentation efficiency; establishing quality control and integrating the whole process with the marketing and distribution infrastructure. Where no marketing infrastructure exists, it has to be created.

Given the progress made by the same Canadian organization in the isolation and injection of pancreatic cells into the spleenic vein (these cells then act as living pancreatic cells capable of releasing insulin in response to the host body's demand), the genetically engineered process of producing human insulin described above may become obsolete in about a decade.

FARMING VERSUS PHARMACEUTICALS

This audience, meeting in an international research centre devoted to agriculture, may feel that I have devoted a disproportionate amount of time to pharmaceutical research and said little about agriculture. My justification is that the high publicity profile enjoyed by biotechnology in general and the many benefits promised by its advocates will inevitably result in intensive competition internationally and nationally for the essential yet very limited resources that are available. If history is any guide, a much greater volume of research and development funds will be dedicated to the health than to the agricultural sectors. The major drug manufacturers invest more than 10% of their sales income on research. The chemical industries allocate between 1% and 3% and the food industries in Canada roughly 0.12% of their respective sales incomes on research. The annual 1983-84 budget of the Government of Canada's Department of Health and Welfare is $21.1 billion, that of the Department of Agriculture is $1.1 billion.

For the industrially developed countries the basic scientific tools of the biotechnology trade are generally widely known. But after a desirable new gene has been stitched into a generally amenable bacterium many production problems need to be overcome before a technologically sound fermentation process is ready for large scale production and commercial exploitation.

The topics to be addressed at this meeting of IARCs at IRRI do not suggest an urgent need to revisit Asilomar. Nor is there need for most of us to carry in our back pockets the relevant provisions of the U.S. Federal Register governing gene transfer among dangerous pathogens. Nevertheless, genetically engineered pathogens for the biological control of prevalent agricultural pests may not be so very improbable in the future. Certainly during the massive invasion of cereal plots by rats in the Sahelian countries a decade ago, a rodent equivalent of myxomatosis would have appeared very welcome.
Recently IDRC engaged a consultant prior to convening a small working group to discuss opportunities for tissue culture research. A copy of the report will be sent to all participants at this IRRI/IARC meeting. The following are a few of the main observations:

Neither tissue culture nor any other of the ingenious techniques lumped under the heading "biotechnology" will foreseeably preempt the established skills of plant breeders, agronomists, pathologists, entomologists, or soil scientists.

Meristem culture could be adapted to a much greater range of tropical plant species. For species of a woody habit of growth, meristem culture remains largely unexplored. Wang and Hu have described a process for potatoes which progresses from the meristem tip via plantlets to more than 30,000 miniature tubers in a period of four months.

In relation to meristem culture the Canadian group recommended more work on genotypic variations, nutrient needs, incubation conditions and cryogenic preservation.

To further exploit callus culture it was recommended, especially for the gramineae and leguminosae, that more attention be given to embryos, to a better comprehension of the genetic instability, chromosome loss, and declining capacity of calli to regenerate over time.

In the realm of protoplast culture, research is needed to understand why transformed cells and the products of heterokaryon fusion often fail to generate viable seed. Are embryonic and juvenile tissues in all instances the most valuable sources of totipotent protoplast?

Given the encouraging experience with Brassica species in Canada, pollen and anther culture offer a potentially effective route to homozygous lines. The problem of albinism is well recognized but the group wondered if "cultural ability" is a heritable trait and whether one might screen cultivars within a species for superior anther culture potential. The group expressed mixed feelings about the applicability of tissue culture to early screening for stress tolerance. The group was not optimistic about early selection for tolerance to drought given its polygenic complexity.

The complete report will be distributed later in the summer of 1984.

RESOURCES AND CONSTRAINTS

The highly favourable publicity given to biotechnology and the many theoretically possible products and processes it offers
suggests to the developing countries an almost limitless abundance of opportunities for better health, more food, sufficient energy, and profitable industrial development and employment. One hopes sincerely that at least a few of these benefits will be realized by those in greatest need.

But much of the literature says little of the resources needed, the investments made, the risks attendant, and the very difficult choices among alternatives for the allocation of very meagre resources. Even the most beneficially endowed nations cannot pursue simultaneously all that biotechnology purports to offer.

Before starting any research and development program, the $64,000 question for all biotechnological companies is: Which market should they choose and which product is going to satisfy the greatest need and give the greatest return on investment?.

It is unnecessary to emphasize to this audience that management consists of choosing priorities among many alternatives, of allocating the physical, material, economic, human and other essential and available resources in pursuit of the priority objectives. For almost all of the developing countries the institutional, industrial, economic and human resources available to them are so meagre that it is not particularly helpful for international agencies or international working groups to offer them a totally indigestible smorgasbord menu of biotechnological abundance.

For most developing countries it is not a question of how many different pharmaceuticals and microbiological conversions can be examined in the laboratory but in what manner can their under-privileged populations hope to gain any predictable benefit from biotechnological research and development. Should they give biotechnological priority to agriculture, health or industrial chemicals? Should they first seek licensing or some other form of cooperative arrangement with established institutions or companies in other countries?

THE LIMITING HUMAN RESOURCE

Though all of their essential resources are strictly limited, the most seriously limiting for most developing countries is a suitably qualified and experienced cadre of research scientists and development technologists. How many of the low income countries with more than 20,000 of population per qualified physician and over 13,000 per qualified nurse, can seriously consider diverting their few medically qualified people from their urgent day to day tasks to biotechnological research and development?
Tables 1-19 show the proportions of new graduates in the various disciplinary sectors in the principal geographic regions of the world. These are total graduates, probably including many at a technical college diploma level among which the proportion of those with research degrees will be relatively small.

Though total numbers are increasing, in most regions of the developing world the proportions in agriculture and natural sciences appear to be declining. Among 50 selected developing countries natural sciences fell from 21.6% to 16.8% of the total graduates between 1970 and 1980. Similarly, the proportion of agriculture graduates declined from 3.7% to 2.9%.

In 1970 Canadian universities graduated roughly 972 people at the PhD level in the natural and physical sciences. By 1980 doctoral graduates in these disciplines had fallen to 872, while arts graduates at the PhD level had more than doubled (Table 19). During the next five years more senior agricultural scientists will retire from Canadian universities and the government service than will be replaced by new PhDs. Though Canada needs roughly 150 new PhDs annually, the eight faculties of agriculture are at present graduating only 70. These trends, evident in other industrialized nations, may well serve to encourage an accelerated brain drain from developing countries.

PRIORITIES FOR THE IARCs

Table 20 shows the public sector investment in agricultural research in various regions of the world. It should be remembered that whereas in most developing countries agricultural research is financed almost entirely by government and development agencies, in the industrialized countries there is a sizeable investment from the private sector.

It is not only the developing countries who will be called upon to make difficult choices from the biotechnological smorgasbord. The IARCs, individually and collectively, are faced with a very thought provoking set of issues. The contribution from the CGIAR members to the IARCs core and special programs have not increased very greatly in recent years. The general decline in donor support of development assistance (apart from the international arms bazaar), together with the relatively low rate of investment in agricultural research in all regions (Table 20), present difficulties for both developing countries and the IARCs. It requires that the IARCs reexamine their priorities and answer some critical questions such as:

1. Who precisely are the clients for the products of the IARCs' research activities?
The general answer is: "National agricultural research systems". But what exactly is the scope and limit of each system and how deeply and broadly can an IARC penetrate or react with each national system?

2. What products of biotechnological research related to agriculture can the various and widely different national systems accept, adapt and use?

3. Will the IARCs develop and demonstrate new and improved techniques of embryo, somatic tissue, pollen and anther culture and if so, to what states of refinement?

4. Will the IARCs pursue the products of these techniques to a stage of improved germplasm that farmers can plant, together with demonstrations of appropriate accompanying agronomic practices?

5. Will the IARCs seek to offer training, technical advice and assistance in biotechnology appropriate to the very many levels of scientific skill and resources among the LDCs?

6. Will the IARCs concentrate first upon the more scientifically and agriculturally advanced of the LDCs - those best able to adapt and profit from new research and advanced technologies?

7. Who will undertake the basic cellular biochemistry and other molecular biology research essential to the greater comprehension and solution of many existing and evident biotechnological problems?

8. Will basic research be subcontracted to universities and/or private research institutes, or will the IARCs do it themselves? In either case, will the IARCs be seeking a significant increase in their budgets or will they be reducing investment in their more traditional breeding and agronomic research programs?

It is worthy of note that a relatively new North American research company is assembling an international team of close to 20 highly qualified biological scientists to pursue advanced techniques for plant breeding devoted almost entirely to two crops. Their horizons for delivery of significantly improved genotypes is a decade into the future.

In the past a vigorous discussion has taken place concerning the IARCs' and LDCs' relations with the International Union for the Protection of New Varieties of Plants (UPOV) and the means by which to protect the IARCs' new crop varieties from restrictive exploitation by commercial seed companies. This is not the time or place to attempt a comprehensive review of proprietary ownership of
novel products or processes of biotechnology. Suffice it to say the whole subject is confused. Some authorities define a patentable variety as one that is new, sexually reproduced, distinct, uniform and stable. The U.S. now permits patenting of asexually generated plants. Several countries permit the patenting of genetically manipulated microorganisms. In the broader context and among different countries the options may include patenting of the product, the process of production, or the end use.

I am told that one of the leading American biotechnology research companies (GENETECH) is reported to have applied for over 1400 patents. Only 80, most for methods of production, have been granted.

IDRC is supporting two relevant projects with the International Centre for Law in Development: (a) Plant breeding and plant breeders' rights in the Third World; and (b) Law and biotechnology. FAO is proposing a major new initiative in the conservation and free availability of essential genetic resources, the scope of which and its potential impact upon the CGIAR family in general and the IBPGR in particular have yet to be determined.

CONCLUSION

It seems readily evident that the potential opportunities and the expectations aroused in human hearts and minds in the short term greatly exceed the resources available to many developing countries for the biotechnological research and development that seems so inviting. The many fascinating opportunities for biotechnological development and the difficulties by which they are constrained will not be realized or overcome purely by laboratory research. The subject is highly complex and deserves a broader spectrum of understanding than it has so far received.

It is hoped that this meeting will stimulate the TAC and the Centre Directors immediately to begin a coordinated and collective plan to determine both short and long term priorities for the IARCs. The plan will need to envisage very precisely what products of biotechnological research within or sponsored by the CGIAR/IARC family will serve the greatest need, and more specifically, whose need is to be served. The priorities, I would suggest, should be directed by careful consideration of what essential needs can be best satisfied and opportunities better realized by biotechnology than by the longer established research methodologies.

Collectively, the TAC and Centre Directors need to determine which essential research can best be undertaken in-house and which requires the direct collaboration of other scientists elsewhere. They will need to offer extensive advice to developing countries about the resources needed and the probable time required to realize any desired objective through a biotechnological process.
Many developing nations will need guidance on the resources they need in order to adapt, modify and apply whatever products of biotechnological research the IARCs propose to offer to them.

More important, the TAC, the IARCs and the CGIAR need to develop a logical, carefully constructed plan for the next decade: a plan which prescribes what the IARCs collectively and individually must pursue, what cooperation with research agencies outside the CGIAR is essential, and the manner in which this cooperation will be developed, coordinated and financed. No mean task, but one that is vital if the promised benefits are to be fully realized.

As a final thought, may I offer a dictum from Sir Francis Bacon: "If we start with certainties we shall end with doubts; but if we begin with doubts and work patiently we shall end with certainties".
TABLE 1.

COUNTRIES INCLUDED IN SURVEY OF GRADUATES
(All data used was 1969/70 and 1979/80 unless otherwise indicated)

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TABLE 2.
GRADUATES/YEAR BY FIELD OF STUDY - SELECTED DEVELOPING COUNTRIES*

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*See Table 1.

TABLE 3.
GRADUATES/YEAR BY FIELD OF STUDY BY REGION

SOUTHEAST ASIA REGION*

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*See Table 1.

TABLE 4.

GRADUATES/YEAR BY FIELD OF STUDY BY REGION

SOUTH ASIA REGION*

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*See Table 1.

**TABLE 5.**

GRADUATES/YEAR BY FIELD OF STUDY BY REGION

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*See Table 1.

TABLE 6.

GRADUATES/YEAR BY FIELD OF STUDY BY REGION

WEST AFRICA REGION*

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*See Table 1.

TABLE 7.
GRADUATES/YEAR BY FIELD OF STUDY BY REGION

MIDDLE EAST REGION*

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*See Table 1.

TABLE 8.

GRADUATES/YEAR BY FIELD OF STUDY BY REGION

LATIN AMERICA REGION*

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*See Table 1.

### TABLE 9.

**GRADUATES/YEAR BY FIELD OF STUDY**

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*Includes Biological Sciences

**Source:** STATSCAN Computer Printout, March 1984
### TABLE 10.

**UNIVERSITY GRADUATES PER MILLION POPULATION**

**SELECTED DEVELOPING COUNTRIES***

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<td>ENGINEERING</td>
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<td><strong>TOTAL</strong></td>
<td><strong>553</strong></td>
<td><strong>1,180</strong></td>
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*See Table 1

World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)
**TABLE 11.**

UNIVERSITY GRADUATES PER MILLION POPULATION

SOUTHEAST ASIA REGION*

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*See Table 1

        World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)
**TABLE 12.**

UNIVERSITY GRADUATES PER MILLION POPULATION

SOUTH ASIA REGION*

<table>
<thead>
<tr>
<th>Field</th>
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<td>238</td>
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<tr>
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*See Table 1

World Population Trends 1950-2000, UN Dept. of  
International Economic and Social Affairs (1979)
TABLE 13.
UNIVERSITY GRADUATES PER MILLION POPULATION

EAST AFRICA REGION*

<table>
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*See Table 1

World Population Trends 1950-2000, UN Dept. of
International Economic and Social Affairs (1979)
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<td>AGRICULTURE, FORESTRY, FISHERIES</td>
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<td>TOTAL</td>
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*See Table 1

World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)


<table>
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*See Table 1

World Population Trends 1950-2000, UN Dept. of  
International Economic and Social Affairs (1979)
TABLE 16.
UNIVERSITY GRADUATES PER MILLION POPULATION

LATIN AMERICA REGION*

<table>
<thead>
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<th>1969/70</th>
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<td>NATURAL SCIENCES, HEALTH</td>
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<td>AGRICULTURE, FORESTRY,</td>
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</table>

TOTAL                      | 325     | 1,146   |

*See Table 1

World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)
TABLE 17.

UNIVERSITY GRADUATES PER MILLION POPULATION

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<td>AGRICULTURE, FORESTRY, FISHERIES *</td>
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<td><strong>TOTAL</strong></td>
<td>3,303</td>
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Source: STATSCAN COMPUTER PRINTOUT - March 1984
World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)

*Includes Biological Sciences
### TABLE 18.

**UNIVERSITY GRADUATES PER MILLION POPULATION**

<table>
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<tr>
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<td>31</td>
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<td>493</td>
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Source: STATSCAN COMPUTER PRINTOUT - March 1984
World Population Trends 1950-2000, UN Dept. of International Economic and Social Affairs (1979)
UNESCO Statistical Yearbook (1975, 1983)
<table>
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<tr>
<th>Level</th>
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<th>1980 No.</th>
<th>1980 %</th>
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<td>71.0</td>
<td>872</td>
<td>50.2</td>
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</table>

Source: STATSCAN Computer Printout, March 1984
<table>
<thead>
<tr>
<th>Category</th>
<th>Agricultural Product Value %</th>
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<tr>
<td>MIDDLE INCOME DEVELOPING</td>
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</tr>
<tr>
<td>SEMI-INDUSTRIALIZED</td>
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</tr>
<tr>
<td>INDUSTRIALIZED</td>
<td>1.5</td>
</tr>
<tr>
<td>CENTRAL PLANNED (Excluding China)</td>
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</tbody>
</table>
THE IMPORTANCE FOR THIRD WORLD COUNTRIES OF A REGULATORY FRAMEWORK GOVERNING BIOTECHNOLOGY

The recent tragedy in Bhopal, India, makes obvious the crucial need for Third World countries to develop effective regulatory and monitoring capabilities to cope with danger otherwise largely unperceived. So far as biotechnology is concerned, the need is even more acute. Much biotechnology R & D has proceeded in an atmosphere of secrecy and such atmosphere is becoming even more intense given the increasing privatization of biotechnology. For some countries in the Third World, the problems resulting from secrecy may be compounded by a lack of present-day technological capacity to fully assess the biohazards they might be importing along with specific products or applications of biotechnology. Usually, Third World countries can rely on determinations as to safety made by regulatory agencies in the industrialized country where the technology was developed, but two special problems arise today. In the first place, there is the policy of the present U.S. government to remove controls it had placed on U.S. corporations regarding the export of hazardous technologies. It is now not only possible but often a deliberate part of corporate policy for U.S. companies to export to the Third World products and technologies which are deemed unsafe and therefore banned in the U.S. A second factor relates specifically to biotechnology. While initially transnational corporation investment in biotechnology was primarily in the field of health (an area which is adequately regulated, at least in the First World), the present investment emphasis is on agriculture (an area which is much less stringently regulated in the First World and is largely unregulated in Third World countries). Moreover, advanced military applications of biotechnology are being developed in the First World and their impacts are of course worldwide. Such military applications need to be closely scrutinized for their potential impacts on the Third World.

There are indications that Third World countries (such as India and Mexico, for example) are adopting the U.S. approach of self-regulation, at least so far as biotechnology R & D are concerned. The approach is increasingly being questioned today even in the U.S. itself. But alternatives to self-regulation do not come easily to Third World countries. In most of such countries, government regulatory agencies tend to be understaffed, overworked and underfinanced. The last-mentioned characteristic has often led to corruption. There is usually very little accountability or regulation of the regulators in developing countries. Consequently, inadequate policing of standards and inadequate law enforcement against violators remain the norm rather than the exception in many Third World countries.

TOWARDS EFFECTIVE LEGAL AND REGULATORY MECHANISMS FOR BIOTECHNOLOGY IN THE THIRD WORLD: SOME ELEMENTS OF A FRAMEWORK FOR POLICY MAKING

So far as biotechnology is concerned, most Third World countries need to address the following questions:

1. why regulate?
2. regulate what?
3. what functions to attribute to the regulators?
4. how to regulate, and the related question: who regulates?
5. how to balance competing interests re regulation and commercialization of biotechnology?
6. how to obtain the information needed to make and implement policies re regulation--what type of compulsory disclosure requirements to adopt?

WHY REGULATE?

Each Third World country will have to decide for itself which of the following interests it wishes to safeguard through regulation:

(a) protection of the environment and community against biohazards—an especially vexing problem relates to communities subjected to continuous exposure, over very long periods, of low levels of potentially toxic substances;

(b) ensuring the safety of the environment of the work place;

(c) protection of the consumers of the products of biotechnology;

(d) protection against the military applications of biotechnology;

(e) curbs on experimentation with human and animal life forms;

(f) curbing the economic exploitation resulting from the introduction of biotechnology (especially through transnationals);

(g) limiting the damage resulting from displacements of Third World crops and products by biotechnology;

(h) curbing the new forms of human exploitation and national dependency made possible by biotechnology.

Obviously, at varying times, there may be strong interests in competition with the above, such as:

i) the promise of biotechnology applications contributing to significant increases in food production to meet the growing gap between food supplies and population growth;

ii) the potential of certain biotechnology processes reflecting significant savings of scarce and expensive energy resources;

iii) adoption of export-oriented-growth-led strategies of national development which would make regulation of any one sector (e.g., biotechnology) unpalatable;

iv) geopolitical and strategic considerations which might be determined to override the interest of national self-reliance by ruling governments.

But even where such interests dominate, it is possible to establish a general regulatory framework and carve out specific and ad hoc exceptions to such framework.
REGULATE WHAT?

The specific laws and institutional arrangements for regulation will depend, in large measure, on what is being attempted to be regulated. So far as biotechnology is concerned, these might include one or more of the following:

(a) Laboratory research: Not surprisingly, this was the first category of activity relating to biotechnology to come under regulation. However, because initially different countries prescribed different regulations and guidelines (and, in some, no restrictions at all were imposed), it was not unheard of for both academic and industrial researchers to undertake their work in other more lenient countries to overcome national restrictions. The U.S. model has been widely followed and, hence, merits some description. In the U.S., the National Institutes of Health (NIH) established a Recombinant DNA Advisory Committee (RAC). RAC created a forum for discussions involving scientists, the public, government and industry. Thereafter, RAC issued stringent guidelines applicable to government-funded research (at the core of which was the requirement that genetically-manipulated micro-organisms be "engineered" so as to be unable to survive (in case of escape) outside of laboratory conditions). Laboratory experiments can be regulated thus by creating categories of experiments, provisions governing laboratory safety and provisions prescribing containment procedures. The NIH/RAC approach used in the U.S. could serve Third World countries, too, as a model for societal decision making on technological risks since the approach permitted discussions and consultations with affected interests before decisions were made, thus paving the way for the necessary public acceptance of developments in the national interest.

(b) Biotechnology researchers: The commoditization of science and the growing industry/university linkages in the field of research on biotechnology have endangered the traditional concept of free access to the results of scientific research being undertaken within an "open global community of scholars". Researchers in most private industries are restricted in the discussion of their work by corporate policies that seek to keep R & D results secret. There is a need for fuller investigation of the mechanisms whereby industry/university collaboration takes place. There are a number of legal and institutional solutions to regulating such contractual arrangements. However, excessive secrecy, withholding publication of findings, refusal to make available strains and vectors relating to published work and restricting access of foreign scientists to research institutions may also result from policies adopted by industrialized countries to protect their international competitive position. There is little that Third World countries can do in the face of such policies except, where effective, to enact their own policies of restriction and permit access to research findings only on the basis of the principle of strict reciprocity. But in this regard, fundamental values and important freedoms of academic life are at stake.

(c) Living organisms: On May 16, 1984, a federal count in the U.S. barred what was to be the first release in history of a man-made organism into the environment. The suit had been brought by environmental activist, Jeremy Rifkin, who asked the court to block the field test. The Committee on Science and Technology of the U.S. House of Representatives, after hearings on potential environmental hazards associated with such release, stated that "while there is only a small possibility that damage could occur, the damage that could occur is great". Special hazards rest in the potential of some microbes to infest higher species and cause diseases. While it is unlikely that such pathogenic microbes
will be used on a large industrial scale, it is likely that experimentation will proceed with certain microbes that affect plants and insects (e.g., Bacillus thuringiensis which is effective against mosquito and black fly larvae). It seems surprising that while pathogenic micro-organisms do present real risks, in contrast to the conjectural hazards of recombinant DNA technology, the latter is regulated while many countries still have no regulations concerning the handling of dangerous pathogens. Ironically enough, although the growth of highly dangerous human and animal pathogens will have to continue for diagnosis, research and vaccine production, recombinant DNA technology is making these products safer than ever before. The U.S. approach is illustrative on the subject. The Environmental Protection Agency (EPA) in the U.S. announced in October 1984 that it would release a broad policy statement for regulating the biotechnology industry in the interests of public health and the environment. But even pending this statement, the EPA has brought into force interim rules governing pesticides developed from genetically-altered microbes. Microbial pesticides are pesticides containing organisms such as bacteria, viruses, fungi, or blue-green algae which have natural pesticidal properties. These organisms are being genetically altered to make them more effective or to broaden their use. Under the interim rules, producers are required to give notice to the EPA before beginning even small-scale field testing of genetically-altered microbes or even when introducing microbial pesticides in natural form into environments where they are not native. If the EPA determines that such testing raises serious environmental or health concerns, it could require the producing company to obtain an "experimental use permit" before it proceeds with the test. The producing company would have to submit more extensive information on the product and agree to very specific controls to reduce the hazards. This represents a significant change in the policy of the EPA because it does not require that such permits be obtained for small-scale field tests of conventional pesticides. Chemicals in such conventional pesticides (unlike their microbial counterparts) have no independent mobility and reproductive capability. Therefore, their potential for causing adverse effects outside the field test site is extremely limited. As the above example shows, there is a crucial need for governments (Third World and First World alike) to play a major role in monitoring testing—even on a very small scale involving the release of new life forms into the environment.

(d) Product testing/market approval: Much the same considerations apply to the testing of other biotechnology products for their ultimate release on the market. Here a careful balance has to be drawn between the interest of rapid commercialization and considerations of public health and the environment. The question of how to ensure a sufficient, timely, and publicly-acceptable review of environmental, safety and health questions needs careful deliberation as the products of biotechnology begin to be commercialized.

(e) Biotechnology production processes: Guidelines and codes of practices need to be evolved concerning pilot-plant and production-scale fermentation of engineered strains. Great care will have to be exercised in implementation because, even if a code were established, for example, re scaled-up work involving the products of genetic engineering, there could be enormous variation from one proposed application to another in terms of competence and experience in operating large-scale fermenters. Public and worker safety must, of course, be the prime concern in designing and operating industrial processes employing biotechnology.
Joint-venture agreements involving biotechnology: In quite a different vein, Third World countries may do well to treat all contractual arrangements for the supply of biotechnology or its products and processes as a separate category of "foreign investment" contract subject to review and approval by a specially-constituted agency distinct from, say, the Board of Investment which reviews all foreign investment. There are obvious justifications for doing so. It seems likely that, for most Third World countries, biotechnology will be accorded "pioneer" or "priority" industry status and, thus, will be offered concessional terms and will be freed from many of the controls applicable to other foreign investment projects. The potential for abuse and exploitation becomes, therefore, much greater. Moreover, biotechnology is a highly-specialized field requiring specialized expertise not necessary for many other types of technology contracts. Biotechnology is also a relatively new field and, therefore, the patterns of contractual relationships (and the pros and cons of different types of such relationships) have not yet emerged with clarity. Until they do, Third World countries may do well to proceed from the basic principle of caveat emptor (let the buyer beware)!

THE REGULATORS

Among the aspects that Third World governments must seek to address quite consciously are the questions: "who regulates?" and "what functions should be invested in the regulations?". We address the former question in the immediately following section because "who regulates" depends in part upon what is expected from the regulators. Any combination of the following functions could be invested:

(a) policy making especially with regard to determination of priorities and balancing of competing interests;
(b) standard setting;
(c) monitoring;
(d) sanctioning; and
(e) public education regarding the priorities chosen and the balancing of competing interests.

Those entrusted with the task of regulating different aspects of biotechnology need to possess not only a broad scientific and technological education but must also possess an adequate appreciation of economic, commercial and social factors involved in the creation of new products, processes and services. It is also significant to note that, for any regulatory agency to perform effectively, it must be vested with:

i) clearly defined jurisdictions;
ii) powers to adopt and work its necessary procedures;
iii) adequate institutional resources and capabilities to undertake timely reviews, if necessary, of even a large number of applications and to act both reactively and proactively.

WHO REGULATES?

Obviously, different approaches to regulation will be needed depending on what is being sought to be regulated. Moreover, conditions will obviously vary
considerably from country to country. Below, we attempt to roster and briefly describe some alternative regulatory approaches, stressing that they are meant to be just that: approaches and not models. So far as regulating various aspects of biotechnology is concerned, an imaginative mix of the following approaches might be possible:

(a) Control Over R & D Funding: This approach might be particularly useful in regulating the direction of R & D in biotechnology. In many Third World countries where government is the major source of funding of biotechnology R & D, this would obviously be an effective entry point into regulation. Even in countries where the government is less directly involved in providing funds for biotechnology R & D, they can still exert influence (through devising tax and other incentives and subsidies) on the direction of biotechnology R & D.

(b) The Self-Regulation Approach: This approach has proved reasonably effective in regulating laboratory research. Self-imposed and self-designed codes of research conduct have, by and large, been respected by the community of scientific researchers. The approach is being increasingly advocated by industry, however, and could be considerably less effective in that sphere. Self-regulation tends to work only when opportunities for conflict between industry self-interest and societal interests are few or when external factors compel industry to adopt a position of enlightened self-interest. This does not appear to be the case, so far, with biotechnology. Moreover, the problem is not substantially different, whether it is private sector or public sector that is attempting self-regulation. There is the view that, in the Third World, regulation may be less important because of the pervasive and dominant role of the public sector in this area. But, as the experience with nuclear energy has shown, it is unwise to feel that assumption by government of a function necessarily obviates or decreases need for regulatory controls.

(c) The Participative-Interdependent Self-Interest Approach: For regulation of certain aspects of biotechnology, especially those relating to health, safety and environment, it may be effective to adopt a "participative" policing approach. Such an approach would involve several concerned interest groups, e.g., workers, consumers, industry and affected community surrounding an industrial plant, participating together in the regulatory agency. The self-interests of each interest group could act as a check and balance of the self-interests of other groups and prevent the abuses of a simple self-regulation approach. Moreover, the conjunction of shared interests might prompt action rather than inaction though, of course, irreconcilable conflicts of interest could lead to a failure of this approach. Obviously, such an approach would be inappropriate where speed is of essence in decision making since participatory decision making by consensus inevitably is expensive in terms of time. But, on the other hand, it may well be argued that certain decisions regarding biotechnology are of such momentous social impact as to require nothing less than participatory decision making by consensus.

(d) The Administrative Approach: This is, of course, the most frequently used approach to regulation: the setting up of an administrative, regulatory agency, empowered by legislation with varying mixes of bureaucratic and technocratic composition. So far as biotechnology is concerned, this approach might be most useful when making decisions of approving products for market release. Here, again, two variants may be possible. One single unified scientific oversight system might be established with power to make decisions re market approval of
all products resulting from biotechnology application. This appears to be the preference of industry in the U.S. But industry concerns are just one among many when it comes to biotechnology. The public interest, the best interest of science, biotechnology and (increasingly in industrialized countries) international competition are all involved. These various interests are by no means mutually exclusive but, also, they are by no means easily reconcilable. Thus, a product-use-oriented-specialized agency approach might be preferable with, for example, one single agency empowered to deal with all products (involving biotechnology or not) pertinent to food and health. Another specialized agency (dealing similarly with products involving biotechnology or not) with an environmental aspect dealing with products relating to toxic substances, pesticides, etc. Issues relating to worker safety and biotechnology-related industrial processes might be well left to the specialized agency with oversight powers over biotechnology research and testing. Some countries (especially where biotechnology is important enough to the economy as a whole) may wish to set up a specialized regulatory agency dealing with economic aspects relating to the biotechnology industry. Such an agency would regulate not only aspects relating to transnational investment in biotechnology but also aspects relating to monopolies and restrictive trade practices in the national biotechnology industry. 

(e) The Strict Liability Approach: It can be argued with some force (especially after the recent tragedy in Bhopal, India) that aspects relating to control over biohazards are important enough to be dealt with by legislation imposing strict criminal liability or tort liability or both, in respect of certain defined aspects of biotechnology-related activities which are to be proscribed. As the Bhopal tragedy so poignantly demonstrates, most Third World countries have yet to begin to explore the usefulness of such a strict liability approach. So far as tort liability is concerned, the industrialized country experience should be borne in mind. Where strict (or even near strict) liability has been imposed, the industry response has been to take recourse to insurance and, more often than not, the costs of such insurance have been passed on, as an intolerable burden to the consumer of the product or service involved.

THE IMPORTANCE OF REGULATION OF BIOTECHNOLOGY FOR THE THIRD WORLD

It is becoming increasingly evident that so far as biotechnology is concerned, Third World countries can no longer place too much reliance on regulation in First World countries to weed out hazardous elements in the export of such technology. In the U.S. and in OECD countries, maintaining an "international competitive position" has become a prime objective so far as biotechnology and all other interests of health, safety and environment, especially in the Third World, will inevitably be sacrificed at the altar of "international competitiveness". Third World countries will thus have to look after their own interests.

Moreover, Third World governments seem likely to come under increasing pressure to turn to biotechnology as an answer to their problems. Part of the pressure will undoubtedly come from transnational corporations whose heavy investment in biotechnology will be prompting them to look now for quick and substantial returns on such investment in the Third World and elsewhere. But, additionally, Third World governments seem likely to face social and economic pressures coming from increasing demand for better health and nutritional
standards, environmental concerns regarding waste management, and increasing costs of oil-based feed stacks and agricultural inputs. As a result, it seems clear that biotechnology will have a major impact in the Third World. In the absence of comprehensive, adequate and effective regulatory frameworks relating to biotechnology, such impact is likely to be more adverse than beneficial for most Third World countries. As one scientist, David Suzuki, puts it, there has been no technology in this century, however beneficial, that has not had a detrimental consequence. I can predict with absolute certainty that there will be a price to pay with biotechnology also.

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NOTES

1. Concern about the regulatory consequences of such a shift of emphasis has been expressed, for example, by Rudolfo Quintero, the head of Mexico's national Biotechnology Program.

2. A major government study of U.S. competitive positions in biotechnology--the "Draft Report by a U.S. Government Interagency Working Group on Competitive and Transfer Aspects of Biotechnology" (Biobusiness World Data Base), Washington, D.C.: McGraw Hill Publications, 1983--makes the following revealing comment on the subject: "America's leadership in high technology industries is the foundation upon which much of our contemporary national security strategy and commercial advantage lies ... It has been a matter of concern that the transfer of biotechnology abroad might jeopardize America's scientific and commercial leadership and national security interests" (p. A-1). The Report goes on to make recommendations for protecting the U.S. competitive position such as: Restrictions for Security Purposes (which contains a section calling for "better control of technical exchange and visits by foreign scientists who have the potential to transfer critical information abroad") (p. A-2); and proposed Response to Foreign Trargeting Practices (that is, the support foreign governments are giving to promote development of biotechnology in their countries).

In the case of biotechnology, as often happens, science has preceded law. The regulatory and legal framework in Canada is one which must be stretched and contorted to accommodate biotechnology research and the production of biotechnologically engineered materials. The need for more appropriate legislation is obvious.

There are at least four areas where biotechnology poses potential health hazards. They are: 1) genetically modified plants and micro-organisms with unknown ecological impacts will be released into the environment; 2) cultures consisting of organisms containing novel genetic information will be handled by many workers; 3) spent organisms will be released into the waste stream; and 4) a new generation of microbial products, such as vaccines, animal growth promoters and food additives will be offered to consumers.

One must keep in mind that these dangers should be of concern both to public authorities, whose job it is to regulate industry, and to industry itself which must be fearful of the civil liabilities it may incur should damage be suffered by anyone in the testing and manufacturing process or by a consumer. For IDRC purposes, there must also be a concern about research funded by IDRC which could result in physical damage to people or to their economic interests.

It is not possible within the scope of this paper to review such laws as may apply to biotechnology in those countries where IDRC might support research involving biotechnology. It is possible, however, to review the state of the law in Canada and, in particular, its shortcomings. It is safe to assume that most developing countries would be no more adequately prepared legally to regulate biotechnology than Canada is.

Canada's situation is rendered more complicated than it otherwise might be because it is a federal state. The Federal Government regulates only environmental matters which are inter-provincial in nature. Both the federal and provincial governments have responsibility in the field of agriculture and can pass laws relating to fertilizers, feed products and pesticides. Waste disposal is the concern of the provinces. Authority for regulating health and safety standards in the workplace is shared, depending upon whether the work is a federal undertaking. To regulate effectively, there will have to be a coordinated approach by the different levels of government.

Dealing first with the question of environmental impact, the Environmental Contaminants Act (ECA), a Federal Statute, was enacted "to protect human health and the environment from substances that contaminate the environment". Under the law, the Federal Government may establish the maximum
quantity or concentration of a substance released into the environment in the course of any commercial, manufacturing or processing activity, and may establish a schedule of substances that may not be imported, manufactured, processed, used, or offered for sale. The statutory language of the ECA unambiguously defines its scope as chemical substances, not biological substances. Presumably, micro-organisms might be used in the manufacture of a chemical substance whose release into the environment is deemed hazardous. In that case, it seems likely that the ECA could be used to regulate biotechnology. However, the Act seems inadequate for the regulation of the release of biotechnological materials themselves.

With regard to the second potential hazard, that is the handling of novel genetic information by workers, both the Canada Labour Code and the "Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells" of the Medical Research Council are relevant. The Canada Labour Code spells out the responsibilities of employers for the use of hazardous substances in the workplace. Dangerous substances are defined as "any substance that, because of a property it possesses, is dangerous to the safety or health of any person who is exposed to it". Although the Act was written for chemical substances and radiation-emitting devices, the statutory language is broad enough to include biological agents or their products should they be deemed hazardous by the Minister of Labour. The Code has no requirements for pre-testing new substances to determine their effect on workers. This evaluation occurs after they have been introduced into the workplace. No regulations or recommendations for the control of substances have addressed the issue of workers' exposure to biological agents or their products. The MRC Guidelines are administered by the Biohazards Committee which comprises four scientists and four laymen. They are written primarily for a laboratory situation where small volumes of culture are used. The situations are evaluated on a case-by-case basis by the Committee. Therefore, there are no special procedures for large-scale recombinant DNA work. These Guidelines are applicable only for research which is funded by the Medical Research Council; however, they do serve as a guide for others who wish to use them.

As for the potential danger posed by new products which are released in the market, one relevant statute is the Canada Seeds Act, which regulates the sale, labelling, importing and exporting of seeds. A genetically engineered seed would be introduced into the system like any seed. A researcher in a plant breeding program could do private field tests but experimental seeds cannot be sold without certification. The most important criteria used in evaluating new seeds are associated with the harvested crop, such as disease resistance and the quality of the grain. It would appear that the two major omissions in the regulation of seeds as concerns biotechnology are the lack of control over field testing and the absence of assessment of new strains with respect to ecological criteria.
There are the tremendous inadequacies in the legal structure in governing both the development and the dissemination of biotechnologies in the marketplace. The development of such laws will be complicated because the hazards of biotechnology have not yet been demonstrated; they are, in one sense, theoretical at this stage. Therefore, designing legislation which is specifically applicable to biotechnology involves more guesswork than is usually the case. Nevertheless, legislators can begin to address the potential hazards in broad terms and one hopes that this will be done sooner rather than later.

I think it is obvious that in IDRC projects which are developed involving biotechnology in developing countries, there will not be much of a legal framework within which our recipients are required to work. Nevertheless, IDRC as a responsible organization must be concerned about, first of all, compliance with such laws as may exist and, second, ensuring that the research which is carried out meets common-sensical standards, legal standards not yet having been legislated. I think we would be risking censure if we were not to insist at least upon those standards which apply to research which is carried out in Canada, or funded research which a prudent researcher would not carry out in Canada for fear of civil liability in the event of anything going wrong.
In any field of science that is developing rapidly, there is not likely to be a well-ordered set of mechanisms for obtaining information about these developments. 'Biotechnology' is no exception. In this case, the difficulties are compounded by a number of factors:

- much of the work that is taking place involves inputs from different 'disciplines' as conventionally defined. Yet many of our better-known and accepted science-information media (e.g. journals for the publication of original research papers) are structured according to these separate disciplines. As a result, much of the information relevant to biotechnology is widely scattered and can be found only by searching through a range of different discipline-oriented information media.

- developments came fast on the heels of each other. Authors and readers often do not wish to wait the 12-24 months that it takes for a paper to be accepted and published by the more traditional refereed journals. Hence, other, more rapid, but less well-known media are employed. Much information first appears in technical reports with privileged circulation, or as contributions to conferences.

- 'Biotechnology' is perceived as an area in which entrepreneurs may be able to make large profits provided they move quickly enough to market new products in advance of their competitors. Some developments are therefore concealed as proprietary secrets, and others will be first reported, not in the normal scientific literature, but as patents. Recognizing the high value attached to some information in this area, another breed of entrepreneurs organizes conferences where attendance is determined by the very high registration fees, or they publish newsletters aimed at an industrial market and with very high subscription prices.

Leading scientists in this area, those that have already earned an international reputation for their contributions, have their own personal networks through which they learn about significant new developments and keep abreast of progress. But it is very difficult for a less well-known scientist to keep up with the field (unless he works for a company that is wealthy enough to send its representatives to the conferences and subscribe to the newsletters). Graduate students have even greater difficulties unless, of course, they are working under a professor that has become a recognized authority and enjoys privileged communications.
Perhaps, most of all, the field presents enormous difficulties for scientists in developing countries who cannot afford to travel or to subscribe to expensive newsletters, or to make international telephone calls. These often have to wait for the late arrival of traditional journals and know that much of what they are reading has already been overtaken by more recent developments.

The field is also exceptionally difficult for those who attempt to set up specialized libraries or documentation centres. To do this effectively needs more than a substantial budget: it also requires the cooperation of scientists who will bring back technical reports from the institutions that they visit, and papers from the conferences that they attend.

Fortunately, the person in charge of one such information service has produced a remarkably useful book, *Information Sources in Biotechnology*. The author, Dr. Anita Crafts-Lighty, is a microbial biochemist and manages an information service within the British biotechnology company, Celltech Ltd. In successive chapters, she guides us through the available textbooks, conference products, trade publications, scientific periodicals, abstracting journals, computerized data bases, patent services, market surveys, etc. She gives good advice to those who are planning to set up new information services in this area.

In each area, Dr. Crafts-Lighty not only identifies a broad range of sources that may be useful, she also homes in on those that she has found to be particularly productive in satisfying the needs of her clients. For example, as in other rapidly moving fields, several publishers are now bringing out series (either annual or irregular) of the Advances in... type. Her lists of these cover several pages but, in her text, she names three that are particularly useful, and another ten that she highly recommends for more specialized topics.

The book was published in 1983. Clearly new sources have appeared since then. In the area of computerized data bases, for example, Dr. Crafts-Lighty identifies four data bases specializing in biotechnology as well as 78 other data bases that are likely to contain significant information relevant to this subject; but Ms. Bev Chataway of the IDRC Library is now able to recommend another specialist data base that was not known at the time when the book was prepared. Dr. Crafts-Lighty's book, nevertheless, remains a basic vade mecum for anyone seeking to find a way through the great variety of different sources from which biotechnology information may be recuperated. We have recently learned that the British Library is undertaking a project to identify biotechnology information sources for the European Community; its product should, therefore, bring up to date what is now available in the 1983 publication.
What could IDRC do about this situation, bearing in mind our mandate to foster research applied to economic and social development? In biotechnology, as in other subject fields, we should pay attention to two aspects of the problem:

- providing a service responsive to the information needs of IDRC staff and IDRC-supported projects

- helping to ensure, through specific projects and other activities, that useful information can be delivered to applied researchers in the developing countries generally.

The principal focal point for providing service to IDRC staff and IDRC-supported projects is the Library in Ottawa. That Library, of course, does not have extensive collections in the field of biotechnology, any more than it has extensive collections in the other more technical subject areas in which the Centre has developed programs. But, over the years, the Library has demonstrated that it can provide service in technical subject areas, by conducting on-line searches in the computerized data bases and by borrowing and acquiring photocopies from other libraries, particularly other libraries in Ottawa. If the Centre develops new program thrusts in biotechnology, it is likely that the Centre's Library can respond and deliver the services needed by staff and projects without making major extensions to its collections, though it may need to subscribe to a few additional "secondary" materials (abstract and review journals). The mechanisms that the Library uses do, of course, demand the dedication of staff resources and, particularly when conducting on-line searches, the staff involved need to have a sufficient understanding of the subject matter and of the clients' needs to ensure that the searches are effective.

To address the larger problem, it is fair to pose questions such as: Should IDRC foster the establishment of a data base to cover information about biotechnology applications relevant to developing countries? Or, should IDRC help establish a library/information service comparable to those that exist in American and European companies (the "biotechnology majors") and make it available to researchers in developing countries? Obviously such things could be done if adequate resources were devoted to them, but they would be expensive and, in seeking to cover the whole gamut of the subjects involved, the cost/benefit ratio would probably be less favourable than for more sharply focussed endeavours.
Traditionally, IDRC concentrates on applied research rather than basic research. Much of what is being done today can be best characterized as 'basic' research, even though many of its sponsors hope that it will eventually lead to profitable applications. However, there is as yet no clear path from much of the present research to the beneficial applications that may come in the future. In the more basic research area, we must recognize the initiative of UNIDO and the establishment of the International Centre for Genetic Engineering and Biotechnology in Trieste and New Delhi. That institution has been created to enable the developing countries to have a window on basic research in biotechnology; inevitably, it will need to have a strong information component to its program.

So, perhaps we should turn our attention to research of a more applied nature, and the information services needed to support it. Here, immediately, we are again confronted with the problem of diversity, for the applications fall into a variety of different sectors -- agriculture, medicine, sanitation, energy, mining, etc. An information service on vaccines for animal diseases needs to be rooted in the realities of animal husbandry as practised in the developing countries; an information service on the use of sulfur-metabolizing bacteria needs to be rooted in an appreciation of the availability of low-grade ores and the potential market for the metals to be extracted; it would be better to consider separate and appropriate institutional homes for information services such as these, rather than to lump them together in an all-embracing information service on biotechnology applications.

This brings us back, therefore, to the concept of the 'specialized information analysis centre (SIAC)' with which the Centre has already had considerable experience. Currently we are supporting about twenty SIACs, each dealing with a well-defined topic and each located in an institution which can itself be considered as a centre-of-excellence for research on that topic. Many of these SIACs deal with individual agricultural crops, but others deal with techniques (use of ferrocement, on-farm irrigation methods). In the biotechnology field, one can imagine support for the creation of SIACs on topics such as "tissue-culture techniques for plants of agricultural significance" or "fermenters for volume production of monoclonal materials".
In fact, there are forces that will work against any efforts in this area. The obvious one is the proprietary protection accorded to some of the most interesting information that is being produced. Another, let it be admitted, is the competitive nature of much of the work in the academic sector, where individual scientists are unwilling to exchange their results before reaching the point at which they can achieve priority in publication (the stuff for which Nobel Prizes are earned).

Many of the problems that now seem very formidable will solve themselves with time. The multiplicity of information media that we now see in "biotechnology" is not unlike what occurred for "nuclear science" in the 1950's and 1960's. But, as in that field, there will eventually be a stabilization and an awareness that certain media have become accepted as the principal vehicles for new information.

The main thrust of this note, however, is to state the thesis that, if the Centre is to be active and effective in this field, it should identify a few well-chosen, specialized topics of priority interest to developing countries, and it should concentrate its resources in trying to build really intelligent information services within these topics. Indeed, it is hardly conceivable that the Centre could make any significant impact if it were to spread its resources over a wide range of diverse topics or to try to find an all-embracing solution to the problem of delivering biotechnology information to researchers in developing countries.

This thesis may be criticized in the sense that it offers only a fragmented approach to a large problem. But we must realize that we are not alone in the field. WHO, for example, has mechanisms for helping developing countries to obtain access to biomedical information. The European Community has recently established a centre to deliver agricultural information to countries of the Lomé Convention. Even if the individual projects we support are narrowly focussed, we can offset this by maintaining our efforts to interconnect the active centres by more effective use of international telecommunications.
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Let a hundred labs bloom.

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Science in India: excellence in the midst of poverty.

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Biotechnology: an ambition and a track record.

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Biotechnology: cooperation can succeed.

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Biotechnology in India.
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Biotechnology and nitrogen fixation.

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The global agriculture support system.

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Biotechnology and the lawmakers.

Biotechnology.

Industrial microbiology.

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Scientific and technology in India, the past, present, and future.

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Priorities for action programmes.

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International Center for Law and Development: evaluation report.

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Genetic engineering: livestock.

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Biomass 2 1982 57-74  
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Biotechnology

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Biotechnology and agriculture.

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Biotechnology in agriculture of the Third World countries.

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Presentation to Nation Advisory Committee on Biotechnology, Canada. June 1984.
Animal health biotechnology in Canada.

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Biotechnology: implications and potential biology pitfalls.

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Elements of some national policies for biotechnologies.

Exchange of view with experts on the implications of genetic engineering and biotechnology on industrialization in developing countries.

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Congress of the United States. January 1984
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Frederick A. Praeger, 1968
Latin America and the Caribbean: a handbook.

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Address at the Central Experimental Farm, Ottawa, Canada. Nov. 1983
Importance of agricultural research in Canada and hence for the developing world.

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New Scientist Feb. 1984 29-30
Industry wants a role for government.

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Biotechnology 2 (1) 1984 55-59
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Bioshelter-based tissue culture: an ecological approach.

* Items not included in Biotechnology file.
BIOTECHNOLOGY ACTIVITIES FUNDED BY IDRC

Since 1981 the Centre has funded 41 biotechnology related activities, nine of which are in Phase II or III. The full list will be found in Annex 1. There have been four recipient countries in Central and South America, three in Africa and 16 in Asia; some of these countries however have had more than one proposal accepted. Eight Canadian universities and four other research organizations have also been involved in these projects.

The biotechnology application breakdown is given in Table I. This breakdown of biotechnologically oriented projects began with the 3-P-81 series, as very few projects were relevant before 1980. Agriculture has received half the support with 19 projects and $3.5 million out of a total allocation of $7 million. The areas receiving these grants were 5 projects on the genetic improvement of crops, 3 each for nitrogen fixation and crop diseases, 4 for aquaculture or fish farming, 2 for pest management and one each for single cell protein production and animal nutrition. The area with the next greatest support is medicine with a budget of $2.5 million; 6 of the total of 11 projects were for therapeutic agents, while there were 2 projects each for vaccines and contraception, and one for diagnostic method. There have been 2 projects for waste treatment for a combined budget of $190,000, and similarly 2 in the biomass field with a total cost of $100,000. There have been no projects under the Industrial sector, but two more sectors are added: "Basic Research" with three projects for $500,000, and "Applications" which has 4 activities 3 of which were symposia, for a total cost of $200,000. These costs are only that of the direct monetary support from IDRC; the person years involved in these activities, which can be considerable, e.g. for setting up a symposium, have not been costed; nor have the contributions of the host country been included.

Fellowships

Over the last four years 39 (or 12%) of the 323 fellowships awarded have been biotechnologically related, with the majority in categories related to projects and programs. There were no fellowships specifically for any "biotechnological" project, so the areas in biology that were counted included aquaculture, but not fisheries; crop improvement, but not forestry; food processing; water purification; and any degree in biological sciences, but not biologically related management, information science or economics.
### IDRC Biotechnology-Related Activities

**AGRICULTURE, FOOD AND NUTRITION SCIENCES DIVISION**

<table>
<thead>
<tr>
<th>Code</th>
<th>Project Description</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-P-81-1001</td>
<td>Microbial protein*</td>
<td>University of Guelph, Canada.</td>
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<tr>
<td>3-P-81-1005</td>
<td>Yellow dwarf virus*</td>
<td>University of Laval, Canada</td>
</tr>
<tr>
<td>3-P-81-1006</td>
<td>Favism research*</td>
<td>University of Manitoba, Canada</td>
</tr>
<tr>
<td>3-P-82-0137</td>
<td>Lignocellulolytic fungi (Phase II)</td>
<td>Mahidol University, Thailand</td>
</tr>
<tr>
<td>3-P-82-0154</td>
<td>Biological pest management</td>
<td>Nagpur University, India</td>
</tr>
<tr>
<td>3-P-82-0198</td>
<td>Fish parasites (Phase II)</td>
<td>Central Research Institute for Fisheries, Indonesia.</td>
</tr>
<tr>
<td>3-P-82-1001</td>
<td>Faba bean pathology (Phase II)*</td>
<td>University of Manitoba, Canada</td>
</tr>
<tr>
<td>3-P-82-1002</td>
<td>Rhizobial carrier systems*</td>
<td>University of Manitoba, Canada</td>
</tr>
<tr>
<td>3-P-82-1005</td>
<td>Fish gamete preservation*</td>
<td>University of Victoria, Canada</td>
</tr>
<tr>
<td>3-P-83-0059</td>
<td>Nitrogen fixing trees</td>
<td>University of Sierra Leone, S.L.</td>
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<tr>
<td>3-P-83-0128</td>
<td>PRACIPA network</td>
<td>CIP, Peru.</td>
</tr>
<tr>
<td>3-P-83-0296</td>
<td>Bamboo (Phase II)</td>
<td>Agricultural Research Council, Bangladesh.</td>
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<tr>
<td>3-P-83-1004</td>
<td>Natural pesticides*</td>
<td>Carleton University, Canada</td>
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<tr>
<td>3-P-83-1010</td>
<td>Fish gametes (Phase II)*</td>
<td>Memorial University and University of Victoria, Canada.</td>
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<tr>
<td>3-P-83-1011</td>
<td>Induced spawning*</td>
<td>China and University of Alberta, Canada.</td>
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<tr>
<td>3-P-83-1012</td>
<td>Dry beans*</td>
<td>Pontificia Universidad Catolica, Chile and University of Guelph Canada.</td>
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<tr>
<td>3-P-83-1031</td>
<td>Genotyping*</td>
<td>CIAT, Columbia and University of Manitoba, Canada.</td>
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<tr>
<td>3-P-83-1032</td>
<td>Tissue culture*</td>
<td>Universidad de Costa Rica and University of Calgary, Canada.</td>
</tr>
<tr>
<td>3-P-84-1006</td>
<td>Leaf spot</td>
<td>Universidad de Costa Rica and University of Alberta, Canada.</td>
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**DAP's**

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<td>3-A-83-2004</td>
<td>Tissue culture*</td>
<td>Wetter</td>
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<td>3-A-83-2018</td>
<td>Soy Rhizobia*</td>
<td>Rennie</td>
</tr>
<tr>
<td>3-A-84-2029</td>
<td>Rumen microflora*</td>
<td>Cheng</td>
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**COOPERATIVE PROGRAMS**

[3-P-83-1015 Enzyme production] University of Punjab, Pakistan and National Research Council, Canada. ] - under consideration.

**DAP's**

<table>
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<tr>
<th>Code</th>
<th>Project Description</th>
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</thead>
<tbody>
<tr>
<td>3-A-83-2050</td>
<td>Symposium on research in biology &amp; biotechnology</td>
<td>Singapore</td>
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<tr>
<td>3-A-83-2079</td>
<td>Support for development of enzyme production project</td>
<td>Pakistan</td>
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<td>3-A-83-2082</td>
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* COOP projects
### HEALTH SCIENCES DIVISION

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<tr>
<td>3-P-82-0155</td>
<td>Anticonceptive technology (Phase III)</td>
<td>India Institute of Medical Sciences, India.</td>
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<tr>
<td>3-P-82-0223</td>
<td>Bilharzia (Phase III)</td>
<td>University of Alexandria, Egypt.</td>
</tr>
<tr>
<td>3-P-82-0225</td>
<td>Yellow fever</td>
<td>Instituto Nacional de Salud, Columbia &amp; Fundação Oswaldo Guz, Brazil.</td>
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<tr>
<td>3-P-82-1008</td>
<td>Bio-control of mosquitoes (Phase II)*</td>
<td>Memorial University of Newfoundland.</td>
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<tr>
<td>3-P-82-1013</td>
<td>Immunodiagnosis of African sleeping sickness*</td>
<td>Trypanosomiasis Research Institute, Kenya &amp; University of Victoria, Canada.</td>
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<tr>
<td>3-P-83-0031</td>
<td>Hospital wastewater</td>
<td>Ministry of Public Health, Thailand.</td>
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<tr>
<td>3-P-83-0156</td>
<td>Piggery waste treatment</td>
<td>Selangor State Veterinary Department, Malaysia.</td>
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<tr>
<td>3-P-83-1006</td>
<td>Sperm inhibition (Phase III)*</td>
<td>Pontificia Universidad Catolica, Chile and Queen's University, Canada.</td>
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<tr>
<td>3-P-83-1029</td>
<td>General eye infections*</td>
<td>University of Kathmandu, Nepal &amp; University of Calgary, Canada.</td>
</tr>
<tr>
<td>3-P-84-0033</td>
<td>Epidemiology of dengue</td>
<td>University of Malaya, Malaysia.</td>
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<tr>
<td>3-P-84-0079</td>
<td>Vaccine trial centre</td>
<td>Mahidol University, Thailand.</td>
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<tr>
<td>3-P-82-0043</td>
<td>Sperm inhibition (Phase III)*</td>
<td>Pontificia Universidad Catolica, Chile and Queen's University, Canada.</td>
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<tr>
<td>3-P-82-0079</td>
<td>Bio-control of mosquitoes (Phase II)*</td>
<td>Memorial University of Newfoundland.</td>
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<td>3-P-82-0043</td>
<td>Immunodiagnosis of African sleeping sickness*</td>
<td>Trypanosomiasis Research Institute, Kenya &amp; University of Victoria, Canada.</td>
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<tr>
<td>3-P-83-0031</td>
<td>Hospital wastewater</td>
<td>Ministry of Public Health, Thailand.</td>
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<td>3-P-83-0156</td>
<td>Piggery waste treatment</td>
<td>Selangor State Veterinary Department, Malaysia.</td>
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<td>Sperm inhibition (Phase III)*</td>
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<td>3-P-83-1029</td>
<td>General eye infections*</td>
<td>University of Kathmandu, Nepal &amp; University of Calgary, Canada.</td>
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<td>3-P-84-0033</td>
<td>Epidemiology of dengue</td>
<td>University of Malaya, Malaysia.</td>
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<td>3-P-84-0079</td>
<td>Vaccine trial centre</td>
<td>Mahidol University, Thailand.</td>
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### SOCIAL SCIENCES DIVISION

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<td>3-P-82-0120</td>
<td>Dissemination of biological nitrogen fixation technology</td>
<td>University of Nairobi, Kenya.</td>
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### INFORMATION SCIENCES DIVISION

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<td>3-A-82-4245</td>
<td>Computer conference on biconversion of lignocellulosics for fuel, fodder and food.</td>
<td>IDRC</td>
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### EXECUTIVE OFFICE

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<td>3-A-84-4005</td>
<td>Safety of novel foods seminar</td>
<td>IDRC</td>
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### SECRETARY'S OFFICE

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<td>3-P-82-0043</td>
<td>Law and biotechnology</td>
<td>International Centre for Law in Development, New York, USA.</td>
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</table>

* COOP projects
TABLE I
IDRC FUNDED BIOTECHNOLOGY ACTIVITIES

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<tr>
<th>Biotechnology applications</th>
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<td>WATER</td>
<td>Hospital waste water</td>
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<td>SEWAGE</td>
<td>Piggery waste treatment</td>
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<td>Rhizobial carrier systems</td>
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<td>Nitrogen fixing trees</td>
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<td>Soy Rhizobia</td>
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<td>Biological pest management</td>
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<td>Natural pesticides</td>
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<td>Bamboo</td>
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<td>Dry beans</td>
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<td>AGRICULTURE</td>
<td>Yellow dwarf virus</td>
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<td>Microbial protein</td>
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<td>Rumen microflora</td>
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<td>Fish gamate preservatives</td>
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<td>Fish gamates</td>
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<td>MEDICINE</td>
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<td>Favism research</td>
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<td>Bilharzia</td>
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<td>Bio-control of mosquitoes</td>
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<td>Corneal eye infections</td>
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<td>Epidemology of dengue</td>
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<td>Virus removal by coal-based solvents</td>
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<td>Immunodiagnosis of sleeping sickness</td>
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<td>Yellow fever</td>
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<td>Biotechnology applications</td>
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<td>FUEL</td>
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<td>INDUSTRIAL</td>
<td>Enzyme production</td>
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<td>Support for enzyme production</td>
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TOTAL
THE STRUCTURE AND REPRODUCTION OF LIVING ORGANISMS

All living organisms are composed of cells. Each cell is basically a physical compartment in which the complex biochemical reactions essential to living organisms take place. The cell is the smallest systematically functional unit of all living things. Cells vary in shape, size and function. The simplest microorganisms each consist of only one cell (unicellular). Viruses may be considered a possible exception since they usually contain only genetic material and some protein. Viruses can exist independently but must invade a living cell in which to reproduce themselves. Higher organisms (eukaryotes) of the plant and animal kingdoms are made up of millions of highly variable cells, each a small factory with specific functions essential to each organism's ability to survive, develop and reproduce itself. Each cell contains a nucleus embedded in a jelly-like mass called cytoplasm. Dispersed through the cytoplasm are various organelles each with a specific function. Organelles include: mitochondria, filaments of protoplasm containing the enzymes that control the cells respiration; ribosomes, particles of protoplasm where enzymes and other proteins essential to the organism's existence are synthesized. Each cell nucleus contains a polymeric chemical substance, de-oxyribonucleic acid (DNA) which specifies and controls the genetic heritage of each cell. The DNA is somewhat akin to a computer program and carries a set of instructions which effectively define and control all cellular activities, all components of the cell and how they are to be made, and the genetic code which carries specific characteristics from one generation to the next. Each cell is surrounded by a thin plasma membrane, a semi-permeable outer layer through which essential substances diffuse inwards and others outwards. Each cell nucleus contains chromosomes, thread-like bodies composed of chains of DNA carrying the genes which control each organism's characteristics. All cells other than sperm, pollen and unfertilized eggs contain two identical sets of chromosomes, one from each parent. As each organism grows and develops, each cell and nucleus divide. Each chromosome also divides so that each nucleus contains a set of chromosomes identical to the cell from which it separated. The number of chromosomes is specific for each organism: peas have 7 pairs of chromosomes, wheat 21, humans 23 and crayfish 100 pairs.

Sperm, pollen and unfertilized egg cells each contain half these numbers of chromosomes (in humans 23 single chromosomes) which are formed by a special process of cell division called meiosis in which the chromosome number is halved. When a human sexual sperm and an egg cell fuse they form a zygote which carries 46 (23 pairs) chromosomes.

The Genetic Code: DNA and RNA

DNA (de-oxyribonucleic acid) is the only living substance that can replicate itself in perfect copy. The DNA copy is passed from parent to offspring and instructs each daughter cell in the animal or plant progeny how to develop and what characteristics to display. Following sexual reproduction, each offspring inherits one DNA copy from the male and a second from the female
parent and thus possesses characteristics from both ancestors. DNA is a very long polymer made up of four different types of very similar organic molecules called nucleotides. Every thread-like DNA molecule consists of two long interwoven spiral chains, each composed of sugar (deoxyribose) and phosphate molecules in a continuous linked alternating sequence. Attached to each sugar unit in each DNA strand is a third component: a purine or pyrimidine base. Each trinucleotide consisting of a sugar unit, a base and a linking phosphate unit is called a nucleotide. The long thin strand constructed of many nucleotides attached together is called a nucleic acid. DNA is composed of two nucleic acid strands wound together in a double helix, each being attached to the other by linkages between pairs of bases like steps in a spiral ladder.

The four bases in DNA are adenine and guanine (purines), cytosine and thymine (pyrimidines). In ribonucleic acid (RNA) thymine is replaced by another pyrimidine base, uracil. For convenience the bases are referred to by their initial letters A, G, C, T and U. The linkages between the bases which hold the two strands of DNA together are very precise and result in the formation of specific pairs. In DNA, A always pairs with T and C with G. As each cell divides to replicate itself, each nucleus divides and the DNA in the nucleus also divides. The cross-linking bonds between the bases are disrupted, the helix unwinds and the two strands of DNA separate. Each DNA strand makes a copy of itself and two identical double stranded DNA molecules are thus derived, one of which passes with the divided nucleus into the daughter cell.

In addition to replicating itself, DNA synthesizes other essential biological substances. Most important is ribonucleic acid (RNA), a chain of nucleotides similar in structure to DNA except that the sugar present is ribose and the base thymine is replaced by another pyrimidine base uracil. RNA molecules are made using DNA as a template. The double strand of DNA separates over a short period of its length and by a process called transcription an RNA chain of complementary base sequence is synthesized. While the alternating sequence of sugar and phosphate is constant in all DNA and RNA molecules, the frequency and sequence of the bases varies. The sequence in which the bases appear along the DNA and RNA molecules provide the code which dictates the pattern according to which enzymes and all other essential proteins will be constructed. Each protein is composed of a specific sequence of amino acids of which there are 20 in living organisms.

The messenger RNA (mRNA) synthesized as described above by the DNA, is used to direct other syntheses in the cell. As stated above, the sequence in which the bases appear in DNA and RNA is highly variable and it is through this variability that the genetic control message is carried and transmitted to the protein synthesis in the ribosomes. Each group of three adjacent RNA bases (called a codon) carries the message for a specific amino acid. Each codon in the mRNA is recognized by another type of RNA: transfer RNA (tRNA) which translates the codon in the synthesis of protein molecules. Each amino acid in
the protein sequence is encoded in a specific sequence of three adjacent bases. For example, in specific instances AAG encodes for lysine; CGA for arginine, AUG for methionine. Other triplet based condons control the start and finish of the process of linking amino acids together as each specific protein is synthesized. A structural gene is the total sequence of condons required to generate a specific protein. It is the central dogma of molecular biology that the information vital to the construction of proteins in living matter is transmitted from the nucleic acid sequences. The message encoded in the DNA and RNA is transmitted to the ribosomes where the protein is synthesized. The mRNA molecule translates the coded message, the tRNA transports and assembles the essential amino acids in the order prescribed by the sequence of bases. When all the essential amino acids are assembled in correct order along the RNA molecule in the ribosome, each amino acid unit is joined to its neighbors by a peptide bond and the whole new protein molecule is unpeeled from the RNA template. The amino acid sequence in each specific protein is precise and apparently invariable. Human haemoglobin contains a sequence of 300 amino acid units. A change of only one amino acid in the sequence leads to sickle cell anemia.

Since the twin chains of DNA are cross-linked through a series of base pairs it is customary to describe the total quantity of DNA present in a cell nucleus in terms of the total number of base pairs. The customary unit is Kb = 1,000 base pairs. Bacteria contain of the order of 5,000 Kb, fungi 50,000 Kb, higher plants and animals between 500,000 and 500 million Kb. The prokaryotic bacteria do not contain nuclei, the DNA is present in the cell in naked strands. The composition and mode of action of DNA in these simpler organisms is less complex than in higher plants and animals which explains why the transfer of pieces of DNA ("recombinant DNA") is better understood and more widely applied in bacteria than in higher plants and animals.

Restriction enzymes present in bacterial cells serve to keep the strain pure by breaking up molecules of DNA foreign to the cell. Some restriction enzymes cut the DNA at random, others at specific points. The restriction endonucleases used in genetic engineering recognize a specific site (nucleotide sequence) within a DNA molecule and cleave the DNA at a particular point within that site. Endonucleases attack within the DNA molecule: exonucleases (present in some viruses which attack bacteria) attack at the end of a DNA molecule removing nucleotides progressively as they (the enzymes) move along the DNA strand. The endonuclease action leaves a "sticky" reactive end to the DNA to which another reactive DNA segment can be attached by other enzymes called (a) terminal transferases and (b) ligases (from the Latin: "ligare" = to bind from which "ligament" and "religion" are derived). An up to date list of restriction enzymes is published annually.

It is thus the function of the DNA/RNA system in living cells to stimulate and control the manufacture of proteins essential to the organism's growth, survival and ability to reproduce. The specific sequences of the purine and pyrimidine bases in the nucleic acid molecules determine the sequence of amino acids in the proteins synthesized. Some of the proteins form the structure, some are stored for subsequent breakdown to produce food and energy for the
growing organism, some are enzymes used to catalyze the synthesis and modification of the carbohydrates, lipids and many other substances upon which the life of the organism depends.

**Genetic Manipulation**

Genetic manipulation (or "genetic engineering" to use the contemporary demotic) is devoted to the deliberate rearrangement of the naturally occurring genetic code as evidenced in the gene sequences along the nucleic acid molecules. The purpose is to rearrange the naturally existing gene sequences or to introduce foreign gene sequences into the naturally occurring nucleic acids in order to confer or enhance particularly desirable characteristics into a living organism. Significant changes in genetic composition and gene expression take place during natural evolutionary processes. The natural process of gene sequence disruption and reassembly is called mutation. Mutation can be stimulated in the laboratory by high energy irradiation or by treatment of the cells with certain chemicals called mutagens. The genetic sequence changes that take place through such mutations are relatively uncontrollable and totally unpredictable. Consequently following treatment with mutagens the organisms have to be grown out and examined to see if any of the mutants are possessed of a change in characteristics desired. Also, during sexual reproduction between two different genotypes of the same species the two different parental genomes may interact and characteristics may be observed in the progeny that are not evident in either of the parents. Heterosis can be simply defined as a significant difference in the mean of any character among the offspring compared with the mean (or mid-parent value) of the same character among the parents.

Contemporary genetic manipulation consists of identifying gene sequences in one organism which control a particularly attractive characteristic and transferring that gene sequence into another organism in which that particular characteristic is desired. So far most practically useful genetic transfers have taken place into simple organisms such as bacteria, the favorite having been *E coli*, an organism which as its name suggests is of a class found in the intestines of animals. It must be stated however that gene sequences found in higher organisms, including the cells of human and other animals, have been transferred to and expressed themselves in the DNA of *E coli*. Since the specific purpose of each gene sequence is to manufacture a specific protein, most of the practical benefits from genetic manipulation has been to induce bacteria to manufacture desirable proteins that are foreign to their normal metabolism. Thus one of the first gene products to be grown in a bacterium was the hormone somatostatin, a protein synthesized in the hypothalamus which acts as a break on the growth of the body. Sufficient publicity has been given in the popular press to the bacterial synthesis of insulin precursors and interferons to require no further comment in this document. Nor is this the place to discourse in any comprehensive fashion on the techniques by which desirable gene sequences are identified, excised and transferred from the DNA of one cell to another. For a comprehensive account of these mysteries readers are
referred to "Man Made Life" by Jeremy Cherfas (Blackwell 1982). Suffice it to say the activity is so intensive and extensive that everything written is likely to be out of date before it is read.

It is worthy of repetition that the techniques by which to transfer foreign genes (pieces of DNA) into simple organisms such as bacteria are more advanced than the means by which to transfer genes between higher organisms. Furthermore, research so far has concentrated upon relatively few bacteria out of the many thousands known and classified about the genetic makeup of which relatively little is known.

Though many microorganisms potentially suitable for use as recipients of foreign genes exist, much remains to be discovered about the genetic makeup of most microorganisms and the mechanisms by which to transfer and stabilize pieces of foreign DNA within their genomes.

Among other limitations the prokaryotic bacteria have limitations in that they produce only "pure protein". Microorganisms such as yeasts are being examined as recipients of foreign genes in the hope of their being manufacturers of more complex biochemical substances. Among many other attendant constraints, it is at present difficult to ensure that the foreign genes inserted become integral and stable components of the recipient host genome; that the desired introduced character is inherited and remanifested in all subsequent generations. Clearly such stability and inheritability is essential in organisms whose natural metabolic functions have been deliberately disrupted towards a technological or economic purpose.

The transfer of foreign DNA into mammalian cells is infinitely more complex and so far only two species: a Simian monkey virus and a mouse virus polyoma have been developed to any significant degree. Similarly, though notable progress has been reported making use of a tumour-inducing plasmid from an Agrobacterium, the transfer of genes between higher plants remains complex and difficult, particularly since ultimately the desired change in character must be stable and transmittable through succeeding generations. An added concern with higher organisms is that the transfer of desirable traits may be accompanied by other unpredictable and quite undesirable changes in character and behavior. Much fundamental research in microbial, plant and animal cell biochemistry is necessary before the many supposed benefits from DNA manipulation promised in the popular media can be realized by humanity.

Genetic Stability

The need to maintain genetic stability within mutated or synthesized strains and to recognize when adventitious genetic mutations have occurred are necessities familiar to all manufacturers of bakers' and brewers' yeasts and those who control other industrial fermentations. To maintain genetic stability in strains which carry transferred foreign DNA may well prove to be much more troublesome at an industrial scale until more is learned about mutagenesis in general and the mechanisms by which organisms repair DNA disrupted by photocatalytic and chemical mutants.
In summary, and clearly subject to legitimate criticism as being an excessive generalization, much that is promised from DNA transfer and genetic manipulation, particularly in systems involving higher plants and animals, remains more speculative than real and calls for immense scholarship, ingenuity and investment in fundamental cell biochemistry and physiology before such processes can be controlled in a truly scientific rather than an empirical fashion.
BIOTECHNOLOGY GLOSSARY

**Adenine:** One of the purine nucleic acid bases present in DNA and RNA. It pairs with thymine in DNA and uracil in RNA.

**Aerobic:** Descriptive of organisms that grow only in the presence of oxygen.

**Amino Acids:** The chemical units of which all proteins are composed. Of the many in existence, only 20 are common to the proteins of living organisms. Of these, 11 are "essential" to humans in that they must exist in the diet and cannot be synthesized in vivo.

**Anabolism:** Chemical changes in living organisms by which chemical energy is stored in chemically complex substances.

**Anabolite:** A substance that participates in anabolism; most commonly an end-product of anabolism.

**Anaerobic:** Descriptive of organisms that grow only in the absence of oxygen.

**Anther:** The fertile segment which surmounts the stamen (the male organ of a plant) and carries the pollen sacs. When the anthers are ripe they open to release the pollen.

**Anther Culture:** A culture of plant cells derived from excised anthers.

**Antibiotics:** Metabolites produced by microorganisms (and probably by higher organisms) which inhibit the growth of rival organisms. The term is particularly applied to isolated, purified and chemically modified substances isolated from cultured microorganisms and employed for therapeutic purposes. Though most widely used to describe anti-bacterials, scientifically "antibiotics" include anti-fungal, anti-viral and anti-parasitic compounds of biological origin. Close to 100 different antibiotics for pharmaceutical applications are manufactured by industrial fermentation.

**Antibiosis:** A state of mutual antagonism between biological organisms (antonym of "Symbiosis").

**Antibody:** A protein manufactured by an organism's immune system to counteract the effect of an antigen. It confers immunity against subsequent infection, in some instances permanently, in others for a finite limited period.

**Anticodon:** The region (base sequence) within a tRNA molecule by which it recognizes a codon on mRNA.

**Antigen:** A substance foreign to a host living organism that stimulates the immune system to generate a corresponding antibody which reacts with and renders the antigen ineffective. Antigens are carried on the surfaces of infecting pathogens. For example the antigens carried on trypanosomes, the protozoa which cause trypanosomiasis are glycoproteins. To protect themselves against the host animal's antibodies, the protozoal antigens continually change by genetic control.
Apical Meristem: The tip of a growing plant root or shoot composed of cells from which subsequent growth develops.

Asexual Reproduction: Reproduction of a plant or animal without fusion of male and female gametes. It includes vegetative propagation, cell and tissue culture.

ATP: (adenosine triphosphate): A substance present in all living organisms. Its conversion to the di- or mono-phosphate liberates energy used for many organic functions including muscular contraction, respiration and "nitrogen fixation".

Attenuation: The reduction or loss of virulence of pathogenic bacteria, fungi or viruses. Attenuated pathogens, in vaccines, are injected into humans and other animals to stimulate antibodies which then confer immunity against future infection by virulent strains of the same pathogen.

Bacteriophage: A virus that infects bacteria.

Bacterium: A major class of unicellular organisms, the smallest living things able to reproduce themselves, achieved mainly by each cell dividing into two. The genetic code is carried in a tangled coil of DNA known as the bacterial chromosome.

Base: A substance which in solution reacts with acids to form salts. Purine and pyrimidine bases are the substances through which the twin helical chains in DNA are cross-linked and the sequence of which in DNA and RNA controls the in vivo syntheses of specific proteins.

Biocontainment: Methods for preventing bacteria and other similar organisms from escaping from the laboratory or processing facility.

Biological Oxygen Demand (BOD): An indication of the amount of oxygen required for the breakdown of organic matter by aerobic bacteria. Specifically used to determine the level of organic pollution of water sources and the oxygen demand in sewage purification.

Biomass: The total mass of material resulting from the growth of an organism, or organisms, plant, or animal. (Note: when a microorganism is grown on a substrate of biological origin, the resultant biomass may include the residual substrate.)

Biopolymers: Long chain molecules synthesized by living organisms. Proteins are polymers of amino acid monomers, cellulose and starch are built from sugar monomers.

Callus Culture: A mass of undifferentiated cells originating from any type of explant. In a callus, usually developed on nutrient agar, the cells are generated in a disorderly unorganized clump, analogous to a pile of loose bricks. In a plant (as are bricks in an architectured building) the cells are differentiated and organized systematically to form shoots, roots and other organs.
Catabolism: Metabolic processes that liberate energy, eg. the breakdown of complex organic molecules by living organisms to liberate energy. (See Metabolism and Anabolism.)

Catabolite: A product of catabolism.

Cell Culture: A group or colony of cells propagated from a single cell in a specifically formulated nutrient medium.

Cell Fusion: The fusing together of two or more cells to become a single cell.

Cellulose: An insoluble, high molecular weight, linear polymer of glucose that is the major component of the solid framework of plants including some cell walls.

Chimera: An organism or piece of DNA constructed from at least two different species.

Chloroplast: A plastid containing chlorophyll. Chloroplasts are the photosynthetic "factories" within plant leaves.

Chromosome: A thread-like body found in cell nuclei, comprised of genes arranged in linear order. In higher organisms chromosomes consist of DNA in association with protein. In bacteria they exist as "naked" DNA. While genes are the units of heredity, chromosomes are the units of transmission from one generation to the next. During cell division chromosomes may break, rejoin or cross over giving rise to new genetic combinations.

Clone: A collection of genetically identical cells or organisms derived asexually from a common ancestor. All members of a clone are identical in genetic composition.

Coding Sequence: The region of a gene that is expressed i.e. translated into protein. Also called an "exon".

Codon: The initial letters of the three nucleotides in the sequence in which they appear in the messenger RNA. Each triplet codon specifies a particular amino acid.

Colchicine: An alkaloid extracted from the root of the Autumn Crocus. It acts, biochemically, to double the number of chromosomes present in haploid cells.

Cotyledon: The embryo leaf in a flowering plant. The Angiosperms (flowering plants in which seeds develop and mature inside a closed ovary) are divided into Monocotyledonae (monocots) with one seed leaf and Dicotyledonae (dicots) with two.

Cytoplasm: The semi-fluid content of each cell which surrounds the nucleus (the nucleoplasm).

Cytosine: A pyrimidine nucleic acid base which pairs with guanine in DNA and RNA.
DNA (De-oxyribonucleic acid): The macromolecular polymer which carries the genetic hereditary message and controls all cellular functions in most forms of life. The twin strands, in the form of a helix, are composed of successive units of the sugar de-oxyribose, phosphate and the bases adenine, cytosine, guanine and thymine, through which the twin strands are cross-linked: adenine to thymine and cytosine to guanine.

Deoxyribose: The sugar present in DNA.

Down Stream Processes: All unit stages in a process (eg. harvesting of biomass, product recovery, separation, purification, storage, distribution, monitoring, etc.) which occur following biochemical change and conversion as, for example, brought about by fermentation. The term is also used to describe the processes and procedures (e.g. pilot plant, manufacturing and distribution) that take place following laboratory research.

Embryo: An organism in its earliest stage of development usually surrounded by protective tissue. The young sporophyte which results from the union of male and female cells in a seed plant (also called a seed germ). An immature organism before it emerges from the egg or the uterus of the mother.

Embryogenic Callus: Callus cultures that under suitable conditions are capable of producing embryos (ie. young plants). Conversely, callus cultures that have lost this capability are termed "non-embryogenic".

Embryo Rescue (also termed embryo capture): When cross-pollination occurs between genetically widely different plants, the resulting embryo may be aborted because of parental mutual incompatibility. Such embryos may be excised and grown on a congenial medium such as nutrient agar. This process is called embryo rescue.

Endonuclease: Nucleases are enzymes that break down nucleic acids into strands of DNA. "Endo" or inside nucleases act at points along the strand and thus break DNA into short pieces. Endonucleases recognize a particular base sequence in DNA and cut the DNA*. Some endonucleases cut the DNA at a specific point; others appear to split the DNA sequences at random. The specific endonucleases are the tools of the genetic engineer who seeks to excise strands of DNA coded for a desirable genetic character. Endonucleases are classed as Restriction enzymes since they are employed, for example, by bacteria to restrict infection by viruses (bacteriophages). The bacterial restriction enzyme attacks the DNA of the infecting organisms. (*see also Palindromic sequences)

Entomogenous Microorganisms: Microbes which infect insects.

Enzymes: Specific proteins which act as biological catalysts to stimulate essential biochemical reactions in all living organisms. Enzymes may be biologically synthesized, extracted and employed to catalyze laboratory or industrial biochemical reactions.

Eukaryotes: A major class of living organisms whose cells possess well-defined nuclei which contain chromosomal DNA (cf. Prokaryotes).
Exon: The region of a gene that is expressed by translation into a specific protein. Also called a "Coding Sequence".

Exonuclease (see also "endonuclease"): Nucleases that attack the nucleic acid strand starting from the ends and thus gradually shorten it are termed "exo" or outside nucleases.

Explant: A small piece of a living tissue taken for the purpose of establishing an in vitro culture. Cell cultures are often identified by the source of the initial explant: e.g., meristem tip cultures, anther cultures.

Fermentation: The process of growing a selected organism, usually a bacterium, mould or yeast, on a substrate so as to bring about a desired change or to generate products of the cells' metabolism (e.g., ethanol and carbon dioxide from yeast fermentation). The term is also used to describe biochemical conversions brought about by isolated enzymes.

Gamete: A mature sex cell or germ cell (in plants the ova and pollen grains), usually haploid in chromosome number, and capable or uniting with another gamete of the opposite sex to form a new plant or animal.

Gene: The linear units of heredity transmitted from generation to generation during sexual or asexual reproduction. In modern molecular biology each gene is a segment of nucleic acid carried in the DNA encoded for a specific protein. More generally, the term "gene" may be used in relation to the transmission and inheritance of particular identifiable traits.

Gene expression: Evidence or manifestation of a genetically controlled characteristic. All of the chromosomal genes in an organism are by no means active at all times. In a plant nucleus as little as 5% of the DNA may be producing protein at any one time. Each organ's system has a unique set of genes, genes that are expressed only in that organ. For example, the petals and leaves of higher plants contain about 7,000 genes which express themselves in a highly specific and regulated manner. Thus all genes may be "active" or "silent" and the manner in which they are switched on and how the "on-off" switches are regulated is yet to be determined by molecular biologists. The process of introducing foreign genes and switching them on in higher organisms is more complex and difficult than in microorganisms.

Gene Mapping: Determining the relative locations of different genes on a given chromosome.

Genetic Code: DNA strands are made up of "triplet codons" (units of three nucleotides) each of which selects for a specific amino acid for inclusion in a protein strand that is being synthesized. The relation between the triplet codons on the nucleic acids and the amino acids in the proteins synthesized is known as the genetic code.

Genome: The entire hereditary message of an organism. The total genetic composition of the chromosomes in the nucleus of a gamete.
Genotype: A group or class of organisms that share a common specific genetic constitution.

Gene Vector: See vector.

Germ plasm: Often synonymous with "genetic material" it is the name given to seed or other material from which plants are propagated. An early theory of inheritance advanced the notion that hereditary characters were contained in an immutable "plasm" transmitted unchanged from parent to offspring (literally (Greek): a plasm is a mould or matrix in which materials may be cast or formed: a "plasma" is the result). A germ plasm bank is an organized collection of seed or other genetic material (each genotype entered being called an accession) from which new cultivars may be generated. In a zoological context germ plasm banks would include collections of preserved sperm, milt or ova and in some cases the animals from which they are derived.

Guanine: A purine nucleic acid base which pairs with cytosine in DNA and RNA.

Halophile: An organism that is tolerant to sodium chloride (salt) in its environment.

Haploid: A cell containing half the number of chromosomes present in the somatic cells. Haploidy is a characteristic of sex and germ cells.

Helix: A spiral coiled form advancing round a central axis like a corkscrew.

Heterokaryon: A cell in which two or more genetically different haploid nuclei may coexist and multiply (also called Polynucleate cells).

Heterosis: An expression of cross-fertilization. A significant difference in the mean (average value) of any character (eg. protein content of a plant seed) in an offspring compared with the mean (mid-parent value) of the same character in the parents.

Heterozygous: An organism that for any given character possesses different genes inherited from the male and female parents.

Homozygous: Organisms which have inherited a given genetic factor from both parents and which therefore produce gametes that are genetically stable.

Hormone: A chemical messenger secreted by the endocrine or ductless glands carried in the blood stream from the gland to a target organ. The hormone induces a specific response from that organ. For example, adrenaline stimulates the heart; auxins and cytokinins in plants stimulate cell proliferation and growth.

Hybrid: A cross between organisms that have different genomes. Hybrids are most commonly formed by sexual cross-fertilization between compatible organisms, but techniques for the production of hybrids from widely differing plants are being developed by cell fusion and tissue culture.
Hybridoma: A hybrid cell resulting from the fusion of a tumor (cancer) cell and a normal cell such as a lymphocyte from the spleen. The fused cells can be cloned and, being derived from a simple spleen cell, will secrete a pure antibody. (see monoclonal antibodies).

Induction: The process that causes a virus earlier inserted into the host cell's DNA to break free and to multiply.

Insulin: A protein hormone produced by endocrine cells (Islets of Langerhans) in the pancreas of humans and other animal species. Insulin, consisting of two cross-linked polypeptide chains, was the first natural protein to be synthesized in vitro. Insulin is essential to adequate glucose metabolism and insulin deficiency causes high blood sugar levels in diabetics. Insulin injections cause a prompt conversion of blood sugar. Insulin was the first protein whose structure and amino acid sequence was determined.

Interferons: Proteins which appear in the blood of mammals following viral infection. Three main types are designated by the Greek letters alpha, beta, gamma, according to their source in the host organism. Their anti-viral mode of action is not fully understood but the amino acid sequences and genetic code sequences have been determined, the latter having been recombined in bacterial DNA to permit production of interferons by microbial cell culture.

Intergeneric Hybrids: Hybrids derived from crossing two species each of a different genus. A viable example is triticale, a hybrid of wheat of the genus Triticum and rye of the genus Secale. The more distant the relation between the two genera, the greater the difficulty of intergeneric hybridization. Many intergeneric hybrids display a mulish infertility, unable to reproduce themselves. More than a century elapsed from the time triticale was first reported and the generation of a fertile triticale hybrid.

Intervening Sequence: In the DNA of higher organisms the active genes (exons) composed of coding sequences which can express themselves in the synthesis of protein are interspersed with other nucleotide sequences (introns) the functions of which are not clearly understood. These non-expressive sequences are called introns.

Introns: The nucleotide sequences in DNA that are not expressed as protein.

In Vitro: Literally "in glass". Experimental reproduction of biological processes in isolation from a living organism.

In Vivo: Biological processes within a living organism.

Karyon: A cell nucleus. Hence Prokaryots and Eukaryots to describe organisms whose nuclei differ in complexity.

Leukocyte: A white blood cell.
Ligase: An enzyme that catalyses the joining together of two molecules. Ligases are used to join strands of DNA in recombinant DNA techniques. ("Ligase" and "religion" are derived from "ligare" (Lat.): "to bind").

Lignins: Complex phenolic plant polymers of variable but undetermined composition.

Lignocellulose: A class of complex substances composed of lignin and cellulose; essential components of woody plants.

Lymphocyte: A type of leukocyte (white blood cell) formed largely in the lymph glands and the spleen which contributes to the body's defence mechanism against infection by its ability to produce antibodies.

Lysis: The physical, chemical or microbiological rupture of a cell wall to release the contents.

Meiosis: The process of division of sexual cells in which the number of chromosomes in each nucleus is reduced to half the normal number found in normal somatic cells. When two sexual cells fuse, each contributes its half of the chromosomes. The resulting embryo contains the full chromosome complement. Cells with half the chromosomes are called haploids: those with the normal chromosomal complement, diploids.

Meristem: The tip of a growing plant shoot or root. A localized group of rapidly reproducing cells at a location of active growth.

Meristem Culture: A cell culture developed from a small portion of the meristem (growing tip) tissue of a plant. Either a stem shoot or root meristem can be used.

Messenger RNA (mRNA): mRNA carries the genetic code for a protein from the DNA to the ribosomes where the code is read and the protein manufactured.

Metabolism: The total sum of the chemical and physical changes constantly taking place in living matter.

Metabolite: A product of metabolism.

Milt: The sperm of fish.

Mitochondria: Filamentous protoplasmic bodies present in cell cytoplasm sometimes called powerhouses of the cell. They carry enzymes which catalyse the biochemical processes of cell respiration and the anabolic conversion of simple substances into compounds which store chemical energy.

Mitosis: The process during somatic cell (i.e. non-sexual cell) division by which the nucleus of each daughter cell contains a set of chromosomes equal in number to the parent cell.

Modification Enzyme: The counterpart of a restriction enzyme. It chemically modifies some of the bases so that the restriction enzyme can no longer cut the DNA.
Monoclonal Antibody: An extremely pure antibody derived from a single clone of an antibody-producing cell. Invading pathogens, viral or bacterial, carry a large number of different antigens each capable of stimulating the host's immune system to generate a corresponding antibody. A single spleen cell exposed to a specific antigen can be fused with a myeloma (cancer) cell. The resultant fused cell, called a hybridoma, continually produces an antibody specifically directed against the antigen. It will therefore seek out and identify the specific antigen. Hybridomas can be cloned and cultured to produce quantities of the pure "monoclonal antibody". Because of its specificity each monoclonal antibody may be used for diagnostic or therapeutic purposes. Most research and development has employed mouse antibodies grown in the peritoneal cavity of immunosuppressed mice. Recently human-human monoclonals have been studied to produce therapeutically useful antibodies for example against tetanus antigens.

Monomer: Chemical units of modest size that combine to form high molecular weight polymers.

Morphogenesis: Literally: the generation of a distinct and organized form. The term is applied to the regeneration of a whole organ or organism from sexual or somatic cells.

Mutagen: A chemical or physical agent which brings about mutation.

Mutant: Organisms, one or more of whose properties differ significantly from the parent organism from which it was derived. An inheritable change in an organism by alteration of the genetic material; a change in the sequence or chemistry of the purine or pyrimidine bases contained in DNA molecules. Mutation can be induced by high energy irradiation or by certain chemical substances.

Mutation: A change in an organism's identifiable characteristics brought about by an alteration in one or more genes.

Mycorrhiza: A symbiotic association between a fungus and a higher plant which increases the plant's capacity to absorb nutrients from the soil.

Nitrogen Fixation: Conversion of gaseous atmospheric nitrogen to an oxidized or reduced form (i.e. NO₂ or NH₃) that can be utilized by plants.

Nuclease: An enzyme which catalyzes the hydrolysis of nucleic acids.

Nucleic Acid: A chain of sugars and phosphates, with a base attached to each sugar. The sequence of these bases make up the genetic code.

Nucleo-proteins: Substances in cell nuclei composed of nucleic acids (such as DNA) combined with a protein molecule. Viruses appear largely composed of nucleo proteins.

Nucleotide: A fragment of nucleic acid strand consisting of one sugar molecule with its attached phosphate and base molecules. Nucleotides link through the phosphate molecules to form nucleic acids.
Nutrient Medium: A liquid broth or semi-solid jelly containing nutrients which stimulate and sustain the culture and proliferation of bacteria, higher plant cells, or animal tissue.

Oncogenic: Cancer producing.

Organelles: Protoplasmic bodies within cells each with a specific function. Types of organelle include nuclei, mitochondria and ribosomes.

Palindromic: Literally reading the same backwards and forwards (e.g. the word “level”). Restriction enzymes can recognize palindromic sequences on twin DNA molecules, for example a particular endonuclease can recognize the following palindromic sequence, where the two strands of the double helix carry the same sequence of bases as in (a) and (b) below:

(a) G - A - A - T - T - C -
(b) C - T - T - A - A - G -

Pathogen: Any disease-producing organism.

Peptides: The chemical linkages by which amino acids are joined together in protein molecules. Molecules (including proteins) composed of a significant number of amino acids are called polypeptides.

Phenotype: The evident and typical characteristics of an organism resulting from the interaction of its genetic expression with the environmental conditions in which it grows.

Phytopathogen: An organism pathogenic to plants.

Plasmid: A small, circular piece of DNA physically separated from and able to reproduce independently of the main chromosomes. Plasmids can replicate themselves autonomously and be inherited by daughter cells. Foreign DNA can be spliced into plasmids and since plasmids can be transferred from one microbial cell to another, even across species, either by bacteriophage vectors or as naked DNA from ruptured cells, they provide the means of genetic transformation: the introduction of foreign genes into microorganisms which express themselves as novel characters in the host organism. Plasmids, incorporating foreign genes are important vehicles for the genetic transformation of organisms.

Plastid: Small dense protoplasmic bodies present in living cells. They are centres of special chemical activity.

Ploidy: The number of sets of chromosomes present in an organism or cell.

Pollen: The dusty, sticky material contained in pollen sacs at the end of the anthers in a plant. When ripe, the sacs split open to release the pollen. Each ripe pollen grain contains two male nuclei equivalent to male gametes.

Pollen culture: A culture of plant cells derived from pollen in a synthetic medium (similar to anther culture). The culture of pollen or anthers on a synthetic medium generates progeny with a single set of chromosomes. A useful means of producing homozygous plants. The single set of chromosomes being doubled by colchicine.
Pollination: The transfer of pollen from the male anther to the female stigma of a flower. Pollen carried between anther and stigma of the same flower is called self-pollination. Pollen carried from the flower of one plant to another of the same species is called cross-pollination. The pollen passes through the pollen tube and the ovary into the ovule where it fertilizes the egg cell. Fusion of the male and female gametes develops in the ovule into a seed consisting of the embryo plant and its nutrients in a protective coating.

Polymer: A macro-molecular substance made up of many repeating smaller units (monomers) bonded together in chain-like sequences which may or may not be cross-linked. Some monomers such as ethylene (the related polymer is polyethylene) are simple molecules; others, such as the nucleotides of which DNA and RNA are constructed, are large and complex.

Prokaryote: Relatively simple organisms such as bacteria that do not contain a distinct nucleus. The hereditary mechanism is controlled by a naked strand of DNA.

Protoplast: A plant cell from which the cell wall has been removed by mechanical or enzymatic means. Protoplasts can be prepared from primary tissues of most plant organs as well as from cultured plant cells.

Protoplast Fusion: Any induced or spontaneous union between two or more protoplasts to produce a single bi- or multi-nucleate cell. Fusion of nuclei may or may not occur subsequent to the initial protoplast fusion.

Provirus: A virus that has become integrated into the host cell's DNA.

Purine: A type of base present in nucleic acids. Adenine and guanine are purines in DNA and RNA.

Pyrimidine: A type of base present in nucleic acids. Cytosine and thymine are present in DNA. Uracil replaces thymine in RNA.

Recombinant DNA: A strand of DNA synthesized in the laboratory by splicing together selected parts of DNA strands from different organic species or by adding a selected part to an existing DNA strand.

Regeneration: Developments of whole organisms from single cell cultures.

Replicon: A DNA molecule capable of replication.

Restriction Enzyme: An enzyme that cuts and effectively excises a piece of a DNA molecule. Some restriction enzymes cut the DNA at specific points, others appear to cut at random. Restriction enzymes, of which many hundreds have been identified and isolated, are important tools in the excision and transfer of specific gene sequences from one organism's DNA to another's. (See also endonuclease and exonuclease)
Ribose: The sugar present in RNA.

Ribosome: Phytoplastmic particles made of RNA and protein. They may be regarded as the assembly units where the polypeptide protein chains are synthesized to the specifications contained in the mRNA code.

RNA (ribonucleic acid): A polymer of the sugar ribose, phosphate, purine and pyrimidine bases which, as an adjunct to DNA, helps to transmit and implement the genetic instructions for protein synthesis carried on the DNA. Some viruses store their genetic information as RNA not as DNA.

Secondary Metabolites: In addition to the primary products of metabolism, the building materials of which living cells are constructed, plants and animals produce a vast range of secondary metabolites, many of which find application in food, pharmaceutical and other industrial technologies.

Serology: The study of sera (plural of "serum").

Serological Typing: A technique based upon antibody-antigen reactions by which pathogenic bacteria are identified. It is particularly useful for strains of pathogens difficult to differentiate by morphological methods.

Serum: The watery liquid which separates when animal blood coagulates. Also the blood serum containing antibodies from an animal previously inoculated with a pathogen or pathogenic toxin, used to immunize human or other animals.

Somaclonal Variation: Somatic (vegetative non-sexual) plant cells can be caused to propagate in vitro in an appropriate nutrient medium. According to the composition and conditions the cells may proliferate in an undifferentiated (disorganized) pattern to form a callus or in a differentiated (organized) manner to form a plant with a shoot and root. The cells which multiply by division of the parent somatic cells are called somaclones and, theoretically, should be genetically identical with the parent. In fact in vitro cell culture of somatic cells, whether from a leaf, a stem, a root, a shoot, or a cotyledon, frequently generates cells significantly different, genetically, from the parent. It appears that during culture the DNA breaks up and is reassembled in different sequences which give rise to plants different in identifiable characters from the parent. Such progeny are called somaclonal variants and provide a useful source of genetic variation.

Somatic Cell: Literally any cell from the "soma" which includes all cells of an organism except the germ cells. In some instances the term is used to describe undifferentiated cells, such as those found in a cultured callus.

Somatic Embryogenesis: The generation from somatic cell or tissue culture of bipolar embryos, similar to sexually derived embryos. Both sexual and somatic embryos possess a primordial root and shoot.

Somatic Hybridization: The formation of hybrids by fusion of somatic cells, as opposed to the fusion of gametes. The term is commonly applied to fusion of plant protoplasts.
Spore: A reproductive body consisting of one or relatively few plant cells. In a congenial medium a spore may produce a new plant.

Sporophyte: A spore-bearing plant.

Stigma: The female organ in a flowering plant. The enlarged distal end of the style on which pollen alights before passing to the ovule.

Style: The elongated thread-like portion of a flowering plant between the ovary and the stigma.

Thermophile: A microorganism that requires or can tolerate relatively high temperature (about 45°C) for growth. Literally: "heat loving".

Thymine: A pyrimidine nucleic acid base which in DNA pairs with adenine.

Tissue Culture: In Vitro methods of propagating cells from animal or plant tissue.

Totipotency: The ability of a cell or tissue to develop into a complete organ or embryo. The capacity of cells for self-differentiation.

Transcription: A process involving base pairing whereby the genetic information contained in DNA puts into appropriate order a complementary sequence of bases in an RNA chain. The synthesis of RNA from a DNA template.

Transfer RNA (tRNA): The molecule that carries amino acids to the ribosomes where an anticodon on the tRNA reads the codon on the mRNA and places the relevant amino acid into sequence.

Transformation: The process whereby a piece of foreign DNA is transferred to a cell thus conferring upon it novel characters.

Translation: The process whereby the genetic code present on the mRNA molecule directs the order of the specific amino acids during protein synthesis.

Transposable Elements: Pieces of DNA that can move from one place to another on one chromosome or move between chromosomes. Sometimes called "jumping genes".

Uracil: A pyrimidine nucleic acid base which in RNA pairs with adenine.

Vaccine: A preparation of a pathogenic microorganism or virus, which has been killed or attenuated so as to lose its virulence but which carries antigens. When injected into a living animal the immune system is stimulated to produce antibodies to counteract the antigens. The antibodies remain in the living system thus providing immunity against any subsequent potentially pathogenic infection by the same organism.

Vector: Literally "a carrier". In genetic manipulation the vehicle by which DNA is transferred from one cell to another.

Virus: The smallest known type of organism. Viruses cannot reproduce alone but must first infect a living cell and usurp its synthetic and reproductive facilities.

Zygote: A fertilized egg.
Antigens

Foreign body injection

Spleen cells each producing a single antibody

Immortal hybridomas each producing a single antibody

Mouse myeloma (bone marrow) tumour cells to confer immortality

Monoclonal cell culture

MONOCLONAL ANTIBODIES