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Technological opportunities in biotechnology

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Foreword

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Abstract

The aim of this report is to present a survey of technological opportunities in a few particular fields of biotechnology: biocatalyst technology, biomaterials, medical diagnostics diagnostics, and pharmaceuticals. The map of technological opportunities has been created on the basis of recent international research findings.

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1 Future opportunities in biotechnology

1.1 The promise of biotechnology

The promise of biotechnology is that it can one day offer an extensive range of products and processes that fulfil a wide variety of human, industrial, and ecological needs. Biotechnology is often being referred to as the next wave of technology, implying that its economic and societal impacts may rival those that are being created at the moment by applications of information technology.

Rather than emphasising the differences between biotechnology and information technology, some authors include genetic engineering in information technologies, as "genetic engineering is focused on the decoding, manipulation, and eventual reprogramming of information codes of the living matter" (Castells 1996: 30). While accepting the statement as partially true, we also have to realise that it is a good example of our tendency to explain various aspects of nature and society by using a particular technology as a metaphor. In the quoted statement biotechnology is referred to as a particular instance of information technology, although there is much more in life than information.

Successful technologies not only transform the economy but also tend to influence the way we see the world around us. It has happened with the simplest of techniques before. Ancient Greeks explained seeing by referring to a table of wax on which a picture is drawn; mechanical innovations of the early Reneissance prompted comparisons of the human body and the universe with intricate clock-work mechanisms; computer processes have inspired thought about the workings of human consciousness. New technologies lead to the creation of new concepts and reveal methods of action that can be used in explaining related and unrelated phenomena. Therefore, in due course, as various applications of biotechnology will become more common, concepts emerging in life sciences and methods used in biotechnology are likely to be used as a metaphors and means in interpreting the world around us. There is all the more reason for this, as some aspects of biotechnology can yield new important insights into the nature of ourselves as human beings and the ecological environment around us.

Biotechnology concerns research, modification and application of substances and processes that have been discovered originally in living organisms. We can define

biotechnology as technical application of knowledge arising from life sciences. Another way to look at biotechnology is to consider its economic promise and see it as “the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (OECD 1982). In the future whole industries will rely on biotechnology. That is emphasised in the definition offered by European Federation of Biotechnology, according to which biotechnology concerns the application of biosystems - cells of microbial, plant and animal origin, parts thereof and molecular analogues - in bioindustries.

While this report is focused on technological opportunities in some fields of biotechnology (biocatalysts, biomaterials, medical diagnostics, and pharmaceuticals), it has to be recognised that there are risks as well. In information technology the year 2000 problem illustrated the risks of misguided programming practices that had quietly penetrated the working habits of a well-educated profession. It can be surmised that in ‘reprogramming life’ solutions could be developed that would later prove detrimental to individual companies and the profitability of the whole sector. The risks of biotechnology include also the spread of bacterial antibiotic resistance, and misuse of personal and familial genetic information. Yet the risks are dwarfed by the rich potential of biotechnology to benefit mankind.

1.2 The biotechnical paradigm of today

Although biotechnology is a rapidly developing field where new important methods are being developed in every few years, a few methodologies have acquired a strong position and can be expected to influence the future development of applied biotechnology. For brevity we shall call these established methodologies the *biotechnical paradigm* of today. A paradigm is a set of methods that experts try first when they seek new solutions or try to understand novel phenomena. The capabilities of research teams, enterprises, and countries depend on their ability to recognise, master and further develop the methodologies of the biotechnical paradigm.

One of the important methodologies used in applied biotechnology concerns *screening*, rapid systematic evaluation of large quantities of compounds, enzymes, antibodies, microbe species, and other targets for a predefined application. Rapid screening assays are available for identifying micro-organisms capable of degrading

specific wastes, for instance. The term ‘high-throughput screening’ has been adopted to depict methodologies that include rapid screening of thousands of compounds. In direct DNA screening DNA sequences of living organisms can be screened by locating genes that code proteins having desired properties.

The second set of methods of the biotechnical paradigm concerns *combinatorial chemistry*. It is used to produce large numbers of modifications of compounds, materials, enzymes, and potential pharmacological agents for later selection. As a whole the process resembles natural selection. As it reduces the need for theoretical modelling studies and yields candidate compounds that can be tested rapidly, the methodology is very efficient in applied research. In practice combinatorial techniques have to be supplemented by modelling and other methods; in general that holds for the methodologies of the biotechnical paradigm as a whole; they form a mutually enforcing set of methods.

The third important methodology in applied biotechnology concerns *molecular modification* or changing the structure of an individual molecule or a gene in a controlled fashion. In developing modification technologies the aim is to enable accurate and yet productive operations at molecular level. While modification may involve only minor changes in the structure of genes, enzymes or other proteins, the resulting changes in the behaviour of the compound can be dramatic. The practical success depends on the extent to which the properties of modified molecules and genes can be predicted by computational or other means. In the future modification methods may evolve into techniques that are useful in nanotechnology as well.

The fourth important methodology relies on the use of *gene transfer* or giving an individual or a species the capability to express a certain protein. In gene therapy the aim is to induce the production of a protein that can remedy a specific medical condition. In the future transgenic plants and animals probably will be used increasingly in the production of valuable proteins, such as pharmaceuticals. In DNA vaccination the host receives a gene expressing an antigen against which immunity is created by the immune system of the recipient. In the future it may also become possible to transfer nonsense genes to cancer cells to treat the disease.

The fifth important methodology arises from *bioinformatics* or the application of computer-based technologies in the study of biotechnology. Computerised methods include those used in building databases on proteins, other molecules and genes; other software methods help in the classification of data contained in databases and

in retrieving and comparing information. Various techniques of molecular modelling are being eagerly developed with one of the goals being the prediction of protein folding patterns on the basis of global optimisation of the potential energy function of the protein molecule. Computer modelling techniques have already proven their usefulness in modelling at the molecular level individual enzymes and their substrates as well as pharmaceuticals and their receptors.

Together these five methodologies constitute the biotechnical paradigm of today. Although the methods are powerful and have produced a great number of successes over the recent years, numerous problems remain to be solved and some basic phenomena are yet to be explained. For instance, the molecular processes of wound healing are not yet known in detail (NIH 1998: 25; Witte and Barbul 1997). The present biological theories tend to describe the effects of a few variables at a time, while the descriptions of entire biological systems and processes remain vague. Many of the processes related to healthy ageing remain obscure. Important insights have been gained on the processes involving neurotransmitters and receptors in the brain, but too little is known about the intracellular processes of the nerve cells. Some virus infections remain difficult to prevent, as it is impossible to predict how the virus evolves. There is similar difficulty in foreseeing the next stages and final outcomes of drug resistance phenomena that are increasingly common among pathogen strains.

1.3 Time frames for realising opportunities in biotechnology

In considering realisation of technological opportunities offered by biotechnology it is useful to distinguish between short, medium and long run time frames. It would be very useful, if we could fix our time frames so that the short run, for instance, would encompass, say, five years. Then we could try to estimate, what is technologically feasible during and after such a time span. The approach would have to be based on detailed evaluations of individual techniques listed in this report. Such a course is not possible here, and instead we shall link the time frames of expected realisation of technologies with methodological advances that are required by the realisation of these technologies.

In the short run the methods used in biotechnology improve hardly noticeably; indeed we shall define short run not as a certain number of years but as a span of time during which biotechnical methodologies applied in products and processes

available in the market place do not improve significantly. What happens in the short run is that some companies succeed in improving products and processes by applying common sense rules, often called heuristics. Seeking cost savings by increasing the volume of production is a typical heuristic; another is to replace a batch process with a continuous mode of production. Improving or tailoring product characteristics, increasing the level of automation, and establishing standards for product inputs are other typical improvements that can be carried over a relatively short time frame of a few years. Companies seeking to apply such common-sense goals usually start by studying carefully their existing products and processes in order to locate opportunities for improvement. The progress achieved is usually incremental, but the cumulative impact over a period of time may be significant. Even marginal advances can be vital in mature fields where cost competition is the prevalent mode of business rivalry.

Table 1.1. Technological opportunities arising in biotechnology in the medium run.

	Biocatalysts	**Biomaterials**	**Medical diagnostics**	**Pharmaceuticals**
Screening	New enzymes, enzyme-producing organisms and genes	New microbial species for the production of biomaterials	Compounds that can be used as indicators for particular targets in diagnostic devices	Compounds that react with particular proteins or receptors
Combina-torial chemistry	New varieties of enzymes with and hybrid enzymes	New varieties of biomaterials	New varieties of indicator chemicals	New varieties of phamaceuticals targeting subsections of population
Molecular modification	Improved efficiency and durability of enzymes	Improved properties of biomaterials; tailored degradability	New varieties of indicator chemicals	New varieties of phamaceuticals targeting genetic polymorphisms
Gene transfer	Improved efficiency of enzyme production	Improved efficiency of biomaterial production	Production of indicator compounds in transgenic species	Production of pharmaceuticals in plants and animals
Bioinforma-tics	Rational methods of enzyme discovery and development	Rational methods of biomaterial discovery and development	Rational methods of indicator chemical discovery and development	Rational methods of pharmaceutical discovery and development

In the medium run companies involved in biotechnology harvest the results of research and development efforts that rely on the biotechnical paradigm of today. The companies bring about new products and processes that are in principle foreseeable today by experts who are familiar with the intricacies of R&D methods of biotechnology. It typically takes 10 to 12 years to develop a new pharmaceutical compound; in other fields of biotechnology the time frame for new product or process development is much shorter. On this basis we can use the methodologies of the biotechnical paradigm of today to describe what kinds of changes are possible in biotechnology in the medium term. Examples of possible future advances in our focus areas of biotechnology are listed in Table 1.1.

Table 1.2. Biotechnology in relation with other major fields of technology.

(Diagonal: feedback of technology upon itself)	**Technologies of materials and structures**	**Mechanisa-tion and electrifica-tion**	**Chemistry**	**Energy technology**	**Automation and information technologies**	**Biotech-nology**
Technolo-gies of materials and structures	Feedback of the technology upon itself (not significant in this case)	Structures and materials of mechanical and electrical devices	Structures and materials applied in chemical technology	Structures and materials applied in energy technology	Structures and materials applied in automation and information technologies	Structures and materials (substances) applied in biotechnology
Mechani-sation and electrifica-tion (incl. micro-electronics)	Mechanisation of materials production and structures manufacturing (building industry)	Feedback of the technology upon itself (not significant in this case)	Mechanisation of chemistry (chemical industry) and electrification of chemicals production	Energy producing engines (energy industry)	Mechanics and electricity (electronics) in automation and information technology	Mechanics and electricity (electronics) in biotechnology
Chemistry	Chemistry of materials production and structural maintenance (prevention of corrosion)	Chemistry of mechanical and electrical, and electronics devices	Feedback of the technology upon itself (not significant in this case)	Energy producing chemical substances	Chemistry of information technology components	Chemistry of biotechnology; molecular biochemistry in particular
Energy technology	Energy technology of materials and structures (structural tensions)	Energy systems in the mechanisatio n and electrification of machines and vehichles	Energy technology of chemical processes	Feedback of the technology upon itself (somewhat significant in this case)	Energy solutions of automation and information technology systems	Energy solutions in biotechnologica l production processes and bioenergetics
Automation and information technology	Automation and information technology in the production of materials and structures	Automation and information technology in mechanical, electrified and electronic systems	Automation and information systems of chemical processes	Automation and information systems of energy production	Feedback of the technology upon itself (very significant in information technology)	Automation and information technology in biotechnology
Biotech-nology	Biotechnology in the production of materials and structures	Biotechnolog y in mechanical and electrified systems (biofilms in processing plants)	Biotechnical systems supporting chemical processes	Bioenergy and biotechnical energy systems	Biotechnology in automation systems (biosensors)	Feedback of the technology upon itself (significant in bio technology)

In the long run biotechnology companies searching for new solutions and business opportunities are able take advantage of techniques that originate from outside of the biotechnical paradigm of today. Research and development teams are sometimes inspired by methods developed in far-away areas of expertise and seek ways to integrate such methods into biotechnology. The result is that solutions from different fields of technology are put together, and the emerging combinations sometimes overcome the limitations of the old constituent technologies. (Sahal 1981: 71.) That is why technical progress is in the long run powered by solutions emerging from combinations of different technologies. On the basis of today's expertise some of the solutions that will emerge in the long run are likely to be surprising and create grounds not only for new products or processes but also enable the development of new markets and co-operation arrangements. Historically biotechnology itself evolved as a combination of microbiology, biochemistry, and industrial production technologies adapted from the chemical industry. Biotechnology and some of its subdomains can be put into a context with other major technologies by juxtaposing them as in Table 1.2. The table shows how new opportunities arise from combinations of various major technologies.

1.4 The limits of opportunities in biotechnology

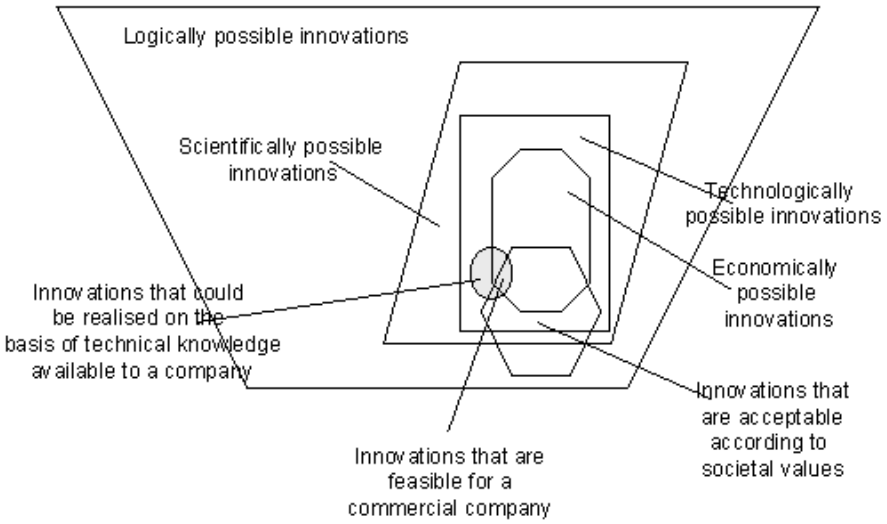
The extent to which technological opportunities can be realised by the present generation of scientists, technologists, entrepreneurs, and financing institutions depends on the available financial, technological, and intellectual resources. Enterprises and research teams can secure access to lacking assets either by acquiring them from the international marketplace or by entering into networking arrangements with holders of the desired resources.

The most pressing limitations usually concern skills; even shortage of funding can often be seen as arising from limitations of skills - those of foreseeing future market conditions and carrying the company's message to financiers. The industrial, commercial and social success of biotechnology depends on the availability of entrepreneurs, financial experts and lawyers, who master the peculiarities of biotechnical operations. To ensure the future availability of such people is one of the main challenges posed by biotechnology to the society. It is very likely that biotechnical expertise is required in the future to a much more larger extent than can

be derived on the basis of the numbers of people who are occupied at the moment in biotechnology either directly or in various supporting tasks.

The role of individual expertise is crucial and well-recognised by the biotechnology industry; yet the risk of demotivation and loss of talent is frequently taken in company mergers. Highly talented people are essential in biotechnology, as competition in the industry is mainly based on technology rather than cost. Most biotechnology companies strive to commercialise new products based on proprietary technologies. Commercialisation involves a wide arrays of skills, as the required tasks include protecting intellectual property rights, ascertaining technical and commercial viability of the product, arranging manufacturing, marketing and distribution as well as obtaining financing. The success of companies in carrying out these tasks profitably determines the rate at which technological opportunities in biotechnology are turned into viable products.

Figure 1 depicts the types of constraints that restrict the development of biotechnology (and other fields of technology as well). The set of innovations that is feasible for a company is but a limited subset of all technological opportunities, and is constrained by economics, regulations and (most of all) R&D skills available to a company.



Source: Lievonen 1998b: 5.

Figure 1. Constraints limiting technological opportunities of a commercial enterprise.

Some of the constraints in biotechnology relate to public perceptions and preferences. While the public seems to accept enthusiastically any proven medical technology, it is much more sceptical about assurances of the safety of genetically modified food products. Once established, public concern tends to seep to public policy, resisting "anything that might kill it, from scientific evidence to official reassurance" (The Economist 1999). In June, 1999 European Union suspended regulatory approval of genetically modified crops, giving another reminder of the fact that the development of a proper regulatory environment for any new technology is a hazardous process.

1.5 Competition and co-operation in biotechnology

Biotechnology as a field is characterised by widespread and all-encompassing networking relations. Small biotechnological companies in particular seek to use outsourcing to carry out non-essential functions; as a result networks emerge in which specialist companies concentrate on biotechnology-related manufacturing, others in marketing, financing, and legal services. In pharmaceuticals, for instance, small pharmaceutical companies, tend occupy an intermediate position in between universities and large pharmaceutical enterprises. (Oliver and Liebeskind 1997.) Universities carry out scientific research and have an essential role in producing new ideas. Small companies perform the first phases of drug development, but the completion of large-scale clinical tests requires the resources and co-operation of a large company.

Because biotechnology is at a phase rapidly technological development, technological turmoil continues and product markets are not always well defined; at any moment breakthrough discoveries can redefine markets. In traditional product markets where competition is mainly about cost there is usually a dominant design for the product. The dominant design is defined as a technological concept that has a market share of at least 50 %; the design has therefore been adopted by major producers in the field. In biotechnology the crux of competition is often the establishment of a particular product as a potential dominant design for the intended use. Companies are not trying to maximise their profits in the short run, but instead strive for medium or long-term success. Perseverance of companies and their financial backers is crucial for the eventual realisation of commercial goals.

As soon as a dominant design emerges, the nature of competition changes. Rivalling companies have to react by incorporating the main features of the dominant design into their products, start cost competition, and the process of industry consolidation may set in. One example of a new dominant design is the first antibiotic drug against the bacterium *Helicobacter pylori* designed for the treatment of gastric ulcers. The new treatment proved much more efficacious, rapid, and economical than previous medicaments. The basis for the new product was established in a breakthrough research that first presented the controversial hypothesis that many ulcers are related to bacterial infection. It took years before the new hypothesis was validated in research and was accepted as a grounds for drug development.

In many fields of technology old established companies, rather than start-ups, have been able to introduce products that have acquired the dominant design status. The strength of established companies is in their technical expertise and close links with customers. Established companies are aware of customers' needs and are trusted by customers. Although young emerging companies are creative and may present technically advanced solutions, they often lack credibility and marketing outlets. Established companies seem to have particular strengths in process innovation, while new entrants have their best chances in product innovation. (Anderson and Tushman 1997: 49-50.) In biotechnology these factors favour networking arrangements between established companies and start-ups.

In biotechnology alliances between new entrants and large companies are common and are likely to benefit both parties. The processes of testing and getting approval to new biotechnical products are complex and expensive especially in pharmaceuticals. Co-operation between start-ups and established companies may help to overcome some of the hurdles on the way. At the same time such co-operation may create risks of new kinds. One of them is that the product market becomes dominated by a few large international companies.

Sometimes new products create new markets rather than introduce added competition to an existing market. While products entering an established market can take advantage of existing chains of distributors and support services, market-creating products may require new social or technical infrastructures. There may be a necessity to establish a chain of distributors that can offer new kinds of services; or the users may require new skills and training; and there may be a need to develop new regulations to manage the risks involved in the misuse of the products. Social infrastructures deal with knowledge (retraining professionals in the field), marketing

(informing potential customers and arranging distribution), and regulation (setting appropriate practices and standards). Technical infrastructures involve technical networks such as transport networks for time-critical materials, communication networks, or information databases and information networks. (Lievonen 1998a.)

The emergence of new markets and infrastructures indicate that a profound technological change is on its way. In information technology the launch of the personal computer created a new market, and the emergence of the Internet created a new infrastructure of global importance.

So far most of the new products that have arisen from biotechnology have been intended to compete with existing products. Relatively few can be seen to have created new markets. Yet the potential for momentous change exists. In the long run we may see, for instance, DNA chips that can rapidly analyse individuals' genetic traits, pinpoint the strains of pathogens that are involved in individual infections, or analyse cancer cells and indicate the best ways of treatment. As the DNA chip technology evolves, it will create a market of a new type in medical diagnostic services. DNA chip technology seems to be of such usefulness and profitability that it will support several specialist manufacturers, a number of specialist supplier companies as well as public and private services. It will also require new information systems and security regulations. The technology makes it possible to produce huge amounts of diagnostic information that needs to be tightly protected from misuse.

Another technological breakthrough of exceptional importance would be the development and widespread adoption of tissue engineering methods for growing organ implants. It is likely that such methods can be used to replace only parts of certain human organs. Even so, the adoption of the methods would require the development of new social infrastructures such as appropriate regulations and methods of training for professionals. Technological infrastructure requirements would include tissue engineering platforms, tissue transport arrangements, and tissue databases.

These two examples of breakthrough products concern human health; in many other fields such as animal husbandry, agriculture, environmental management, and processing industry biotechnology is going to create opportunities for radical and profitable innovations. The next few chapters present a survey of technical opportunities that can be discerned on the basis of recent research.

2 Biocatalyst technology

2.1 Defining biocatalysts and enzymes

In this report the terms ‘biocatalyst’ and ‘enzyme’ are used almost interchangeably as synonyms. Yet it should be kept in mind that biocatalysts encompass a wider technological scope than enzymes, which are a special type of biocatalysts consisting solely of proteins. In addition to enzymes biocatalysts include a wide variety of compounds and microbes that have a catalytic effect on particular reactions or processes.

Biocatalysts and enzymes can be defined as catalysts of natural origin stimulating or precipitating chemical reactions in living organisms or industrial processes. Enzymes are natural proteins, and they can be modified, built as hybrids from existing components or designed *de novo* by using various protein engineering methods. Some of the biocatalysts of the future will be synthetic compounds that are to be used to replace enzymes for reasons of cost or stability.

Most enzymes catalyse only one reaction. One enzyme can accelerate the synthesis or the breakdown of a particular substance, known as a substrate of that enzyme. Being specific to particular substrates (or bonds), most enzymes produce little or no by-products and are therefore efficient and environmentally friendly. As most enzymes are of natural origin, they operate mainly in mild and aqueous conditions that are environmentally benign but also conducive to the growth of harmful micro-organisms and fungi. Like catalysts enzymes remain in principle unaltered in the reactions they govern, but in practice they are gradually inactivated and have to be replenished. Enzymes are prone to inhibition by various substances, to degradation, and to denaturation. All of these phenomena restrict the duration for which a dose of an enzyme is useful, measured by the half-life of the enzyme.

If a biocatalyst is available for a given reaction, its application increases the rate of reaction by a factor of between 10^6 and 10^{24} (Benkovic and Ballesteros 1997: 385). The number of known enzymes is usually estimated to be about 3 000, but the number of commercialised industrial enzymes is only a little more than one hundred. Proteases (for detergents, cheesemaking and other applications) are commercially the most important group of industrial biocatalysts of today followed by carbohydrases (including amylases, glucose isomerases, pectinases and cellulases) (Uhlig 1998: 10).

While some references will be made to the application of biocatalysts in biochemical research, pharmaceuticals and medical diagnostics, our focus is on industrial enzymes and production technologies based on biocatalysts. As will be shown shortly, biocatalysts will offer business opportunities not only for enzyme producers and user industries but also for companies of high expertise specialising in support services of various kinds.

2.2 Biocatalyst markets and technologies of today

Towards the end of the 1990's the annual value of the world's industrial biocatalyst market was about $1.5 billion and was growing at the rate of 3-5 % a year (Cultor 1999). The main segments of the market were technical biocatalysts (enzymes used in the chemical and paper industries) as well as food and feed enzymes. In the marketplace biocatalysts compete with traditional chemical synthetic technologies. As enzymes are relatively expensive, they are usually not suitable for the production of bulk products, unless environmental concerns weigh heavily in their favour. Table 2.1 lists some types of enzymes and their substrates, reactions they catalyse, and industries in which they are applied.

Well-established uses of biocatalysts include the application of glucose isomerase in the production of fructose, rennin in cheese making, cellulases and lipases in detergents, and the application of various enzymes in the synthesis of amino acids, peptides and antibiotics. Other success stories include enzymes for starch utilisation, animal feedstocks, and pulp and paper industry. Most of the commercial biocatalytic processes of today involve bond cleavage through hydrolysis or bond formation by the reversal of hydrolysis. Industrial applications of reactions forming carbon-carbon bonding can be found mainly in the synthesis of sugars and amino acids. (Lalonde 1997.)

The use of the thermolysin in the production of aspartame in an ambient temperature is one example of a process in which enzyme technology presents considerable economic benefits. In addition to energy savings enzyme technology offers high specificity. However, as many enzyme producers operate in a buyers' market, it is the user industries, instead of the enzyme manufacturers, that are able to expropriate a lion's share of the economic benefits that biocatalysts offer.

Table 2.1. Some types of biocatalysts, their substrates, reactions they catalyse, and industries in which they are applied.

Type of enzyme	**Substrate**	**Reaction catalysed by the enzyme**	**Industry**
Industrial enzymes			
Proteases (proteolytic enzymes)	Proteins	Proteins are broken into shorter fragments, peptides and eventually into amino acids	Detergents, food, pharmaceuticals, chemical synthesis
Carbohydrases	Carbohydrates	Hydrolysis of carbohydrates into sugar	Food, feed, pulp and paper, textiles, detergents
Lipases	Fats (trigly-cerides)	Hydrolysis of fats into fatty acid and glycerol molecules	Food, effluent treatment, detergents
Pectinases	Pectins	Clarification of fruit juices	Food, beverage
Cellulases	Cellulose	Hydrolysis of cellulose	Pulp, textile, feed, detergents
Amylases	Poly-saccharides	Hydrolysis of starch into smaller carbohydrate molecules such as glucose and maltose	Food
Enzymes for research applications			
Restriction enzymes	DNA	Breaking DNA at specific sites of the DNA chain	Gene technology
Ligases	DNA	Repairing damaged DNA, joining pieces of DNA into plasmids	Gene technology
Ribozymes	RNA	Ribozymes are molecules of ribonucleic acid that have catalytic effects	Gene technology, virus research, pharmaceuticals
Abzymes	Various	Abzymes are antibodies having catalytic effects	Pharmaceuticals

2.3 Future opportunities in biocatalyst technology

2.3.1 Technological opportunities - supply of technologies

Biocatalysts provide means to manufacture a great number of substances. In principle all compounds found in living organisms are products of enzymatic processes. At the moment only a small proportion of such compounds can be produced economically by utilising enzymatic processes on an industrial scale.

At present the discovery and definition of a new enzyme is a time and resource consuming process, but various technologies are being developed to facilitate the process. At present new enzymes are sought by applying computerised database methods, robotic systems, and miniaturised high-throughput screening systems. In the future it may become feasible to design enzymes for special purposes by using combinatorial and molecular modelling techniques. Efforts are being made to develop and apply *de novo* design and synthesis of catalysts from organic molecules, such as macrocyclic compounds, polymers, cyclodextrins, and peptides.

New enzymes can be constructed by combining parts of others. Sometimes it is possible to create a hybrid enzyme by joining segments of two genes coding two enzymes. The resulting hybrid enzyme can display attractive characteristics, such as broad substrate specificity, making the use of the enzyme more practical. (Hopfner *et al*. 1998.) A futuristic conjecture would be to express hybrid molecules consisting of the active site of an enzyme and a suitable interface protein that would enable the immobilisation of the enzyme molecule on a preferred surface. Such a method could be used to produce inexpensive enzyme detector components for miniaturised diagnostic devices.

Trends concerning enzymes, substrates, and reaction conditions are summarised in Table 2.2. The table represents what we call foreseeable technological opportunities in enzyme technology. For the sake of simplicity techniques requiring regulatory enzymes, cofactors (metal ions or coenzymes) or inhibitors are not considered.

It is likely that new powerful enzymes will be located in the *biochemical energy, nitrogen, and iron cycles* of nature. For instance, in cellular mitochondria an enzyme called cytochrome c oxidase breaks up tightly bound oxygen molecules and combines them with hydrogen atoms to make water. The resulting ion flow provides energy needed in transforming adenosine diphosphate (ADP) into adenosine triphosphate (ATP) that acts as an energy source for many cellular processes.

(Stanford University 1997.) As methane is an important industrial raw material and energy source as well as a potent greenhouse gas, biotechnical processes related to it are of considerable importance. All methane-producing bacteria use an enzyme called methyl-CoM reductase. The crystal structure of this enzyme has been solved recently to a high resolution. (Max-Planck-Gesellschaft 1997.)

Table 2.2. Foreseeable technological opportunities in enzyme technology.

Enzyme and substrate technologies	**Reaction conditions (and examples of future applications)**				
	Temperature			**Pressure**	**Solvent**
	-2°C ≤ T ≤ 5°C	5°C ≤ T ≤ 42°C	42°C ≤ T ≤ 110°C	high/low	aqueous/non-aqueous
Screening microbes and DNA for new enzymes					
- high throughput screening - direct screening from uncultivated micro-organisms	from cold habitats (for environmental applications)	from normal habitats (for medical and industrial applications)	from hot habitats (for processing and chemical industries)	from undersea/ surface habitats (for processing and chemical industries)	from natural/ laboratory or industrial conditions
De novo design of enzymes					
- rational design methods - directed evolution - hybrid enzymes	design of enzymes for low temperatures (for food industry)	design of enzymes for extraordinary substrates (for unorganic substrates)	design of enzymes for hot temperatures (industrial applications)	design of enzymes for high pressure conditions (industrial applications)	design of enzymes for non-aqueous solvents (industrial applications)
Modification of existing enzymes					
- chemical modification - recombination techniques - combinatorial chemistry - directed evolution - hybrid enzymes	modification for lower temperatures	modification for efficiency	modification for higher temperatures	modification for different pressure conditions	modification for different solvents
Enzyme product preparation					
- bulking - coating - preserving - immobilisation - cross-linked crystals	improving enzyme properties at low temperatures	improving enzyme properties	improving enzyme properties at high temperatures	improving enzyme properties at high pressures	improving enzyme properties for different solvents
Modification of substrates before enzymes are applied					
- chemical methods - recombination techniques (proteins) - physical methods (heating, cooling, etc.)	increasing efficiency of enzyme reactions at low temperatures	increasing efficiency of enzyme reactions	increasing efficiency of enzyme reactions at high temperatures	increasing efficiency of enzyme reactions at high pressures	increasing efficiency of enzyme reactions

The promise of high-throughput screening and combinatorial chemistry is strengthened by the emerging microreactor technology. The former two methods are useful in locating new enzymes and in improving their properties; microreactors can be used to test the viability of new enzymatic processes. In microreactors miniaturised mixers, catalytic reaction chambers, and separation devices are integrated for the performance of test runs that approximate what could happen in full-scale systems. (Fairley 1998.) Microreactors may have a particular relevance for companies that lack pilot plant resources.

2.3.2 Technological requirements - demand for new technologies

The main users of biocatalyst technology can be divided roughly into four segments. Industries manufacturing goods in bulk quantities constitute the first category; this segment includes also companies that apply biocatalyst technologies in the waste treatment business or in the remediation of environmental problems such as oil and chemical spills. The second set of biocatalyst users consists of producers of fine chemicals; in their line of business production volumes are relatively small but prices high. The third segment consists of the users of enzymes in medical, biological and biotechnical research as well as in medical, environmental and industrial diagnostics; the amounts of enzymes they require are small but the prices of some substances are extremely high. The fourth type of demand concerns not biocatalyst technology directly but expertise related to it. Services based on biocatalyst technology expertise are provided by companies specialising in designing chemical and biotechnical processes for various sectors of industry.

The four customer segments of biocatalyst technology obviously overlap to some extent and are subject to change over time. An expensive fine chemical of today may be a bulk chemical of tomorrow. Companies manufacturing bulk commodities may require the services of specialist engineering design companies that may in part rely on expertise related to biocatalysts. The four market segments will influence the future demand for biocatalyst technologies and warrant further discussion.

2.3.3 Demand for new biocatalysts in the production of bulk materials

Among bulk commodity producers the main determinant of biocatalyst demand is the relative cost. In bulk commodities markets tend to be mature; competition and experience that has accumulated over time have ironed away most technological differences between manufacturers of bulk commodities. The competition is mainly price competition, and the supplier industries face a strong pressure to reduce costs as well.

When biocatalysts are required by bulk product industries, the requirements are almost automatically huge allowing biocatalyst producers to implement improvements in production methods. That is why the prices of enzymes used in bulk commodity industries tend to decline. One way to achieve cost reductions in enzyme production is to introduce efficient microbial production processes. Enzyme producers often find that competition forces them to pass the cost benefits downstream towards the final customer. The cost competition may also restrict the extent to which it is economically feasible to develop new enzymatic processes. Even when efforts to develop new enzymes targeting bulk substrates have been technologically successful, the results have not always been commercialised. The relatively high cost of enzymes can be justified in cases where environmental considerations or the need to reduce the amount of side products favour enzymatic methods. As a result of painstaking research some of the problems related to costs can be expected to overcome, and enzymatic methods can be expected to make slowly inroads into the production processes of industries using bio-originated materials in bulk quantities.

In some cases side products of production processes involving bio-originated materials can be turned into valuable speciality products such as ingredients of fine chemicals, feedstocks, or functional foods. Successful xylitol and plant sterols products demonstrate the potential of developing side products of bio-originated bulk materials. Xylitol is an anticariogenic sweetener; it is produced from acid-treated birch wood fibres. In the future xylitol may be also produced from xylan of, for instance, corn fibre. It may also become possible to develop an economically viable microbiological production process, since many yeasts and fungi synthesize an enzyme that catalyses the reduction of xylose into xylitol. (Silva *et al.* 1998.) Valuable substances derived from side products of conventional processes constitute an intermediate product class between bulk products and fine chemicals. For such intermediate products enzyme technologies enable the development of

highly specific and pure production processes. Being particularly versatile in treating substrates of natural origin biocatalysts may find a number of applications in the production of flavours, pharmaceuticals and special nutrients based on bio-originated materials.

For industries producing wood and paper products enzymes that can be used to modify or degrade cellulose or lignin are of particular importance. Extensive studies of enzymes produced by *Trichoderma reesei* have already been carried out. The intestines of wood-eating termites, of which there are hundreds of species, may contain microbes and fungi that produce interesting enzymes. (Brune 1998: 20.) Methods to save energy in mechanical pulping could offer significant economic benefits; in the future the use of biocatalysts may offer a partial solution to the problem. If economically viable enzymatic methods for cellulose hydrolysis could be developed, cellulose from wood or even grass could become a source of fermentable carbohydrates for food and fuel production (Uhlig 1998: 89). Processes combining enzymatic treatments and engineered bacteria are being developed in order to enable production of ethanol from cellulose (Ingram *et al*. 1998).

In the food industry new biocatalysts may be used in the future to enhance the quality of products and to preserve them from microbial contamination. In one study transgenic mice secreted the human lysozyme enzyme in their milk; as a result the milk was found to be bacteriostatic against a mastitis-causing strain of *Staphylococcus aureus* but not against a pathogenic strain of *Escheria coli*. (Maga *et al*. 1998.) Future packaging materials may contain enzymatic ingredients that have antimicrobial properties or improve the quality of the product. One candidate for flavour enhancement is naringinase, which reduces tartness of grape fruit juice. The effect can be accomplished by coating the inside surface of the paperboard carton package with a cellulose polymer impregnated with the enzyme. (Andreeva 1998.)

As enzymes are natural products, they are environmentally friendly and well suited for applications in environmental technology. The demand for new enzymes can be expected to grow in the treatment of liquid industrial effluents, sewage waste, and possibly even in water purification. Immobilised horseradish peroxidase has shown promise in the treatment of effluents from paper and textile industries (Peralta-Zamora *et al*. 1998). The strive towards reduction or even elimination of waste creates important opportunities for biocatalyst technology, particularly so in

industries using large quantities of bio-originated materials and in those involved in the production of fine chemicals.

There is some potential for biocatalysts also in the reduction of environmental impacts of energy production. Some bacteria may be useful in the desulphurisation of coal, crude oil, and oil distillates. In one experiment up to 30 % of the total sulphur in the middle distillate cut was removed, and another 30 % was oxidised indicating the onset of the desulphurisation process (Grossman et al. 1999). In the future it may become possible to develop efficient enzymatic treatments that can be used to remove sulphur from low quality fuels. Even partial success would be useful in upgrading the fuels and reducing sulphur emissions. Similar progress may become possible in removing heavy metals from fuels and other industrial raw materials. In addition to cost, the main problem in flue gas scrubbing is the low thermal stability of most enzymes. Oil and coal are also important sources of fine chemicals, and biocatalysts offer important opportunities in this sector as well.

2.3.4 Demand for new biocatalysts in the production of fine chemicals

In the future production processes of fine chemicals can be expected to offer opportunities for the application of biocatalytic processes. As many fine chemicals are relatively highly priced, the use of biocatalysts is not precluded of the basis of cost considerations. Fine chemicals include intermediate products required in the manufacturing of pharmaceutical compounds, paper chemicals, high-quality pigments, flavours and fragrances. Altogether these products comprise thousands of valuable compounds. In fine chemicals the quality of intermediate products and processes is of extreme importance. As the end products of biocatalyst reactions are very pure and specific, biocatalysts are well suited for this segment of industry.

The production processes of some pharmaceuticals and fine chemicals generate several hundred times more waste than the actual product. There is always room for new improved processes that can either reduce the amount of waste or help in its treatment. A related problem in the production of fine chemicals is the large number of byproducts. That is the case in the conventional method of producing acrylamide, a compound used in sealants. In order to obtain enzymes that could turn acrylonitrile into acrylamide, a large number of microbes were screened. A superior microbial strain was discovered and enabled the establishment of a simple and clean process

without unnecessary byproducts. (Ogawa and Shimizu 1999: 14.) A Japanese plant applying the method has a capacity of 20 000 tonnes a year (Hunter 1998).

Enzymes degrading practically any organic chemical compound, including DDT, can be found when a full range of bacteria, fungi and yeasts are screened in sufficient detail. (Bonaventura and Johnson 1997.) After a particular enzyme has proven efficient, molecular probes can be used to test mixed microbial populations for genes that code for that enzyme. In the future the cost of using enzymes in environmental applications could be reduced if it became possible to adopt evolutionary methods, in which large numbers of species of microbes are cultivated in laboratory conditions that resemble the site that needs to be cleaned. Combinations of enzymes and microbe species could be found that would produce acceptable remediation results even if the details of the process would remain indeterminate.

Chiral compounds play a rapidly increasing role in fine chemicals used in pharmaceuticals, pesticides, and flavours. Enzymes are able to differentiate substrates according to chirality; such stereospecificity is a characteristic feature of all natural proteins. That is why living organisms sometimes react with a marked difference to the two chiral forms (enantiomers) of a substance. One harmless example is aspartame; one of its enantiomers tastes sweet, while the other has a bitter taste.

Small amounts of immobilised enzymes are required for biosensors and other diagnostic devices; the combination of oxidoreductases and amperometric electrodes is often the most promising solution for biosensor applications. Sensors of the type are already available for about 80 substrates. Although it can be argued that the promise of biosensors has been overrated, the search for suitable new enzymes for them continues. (Luong *et al*. 1997.)

2.3.5 Research applications and technology services based on biocatalysts

In research and laboratory applications enzymes have many advantages because of their high specificity and mild reaction conditions; in many areas of biological research enzymes are indispensable. In the research market segment the users of biocatalyst technologies have high skills; another special feature of this market

segment is that most of the customers can be reached via the Internet, which may substantially reduce the costs of marketing and logistics. Consequently it may become viable to commercialise products that have relatively few users in different parts of the world.

Biotechnical and biomedical research activities are intensifying at a rapid pace in many industrialised countries. As a result, companies specialising in laboratory enzymes are facing good prospects for growth. The danger is that price erosion may be rapid in some product segments, and the producers have to be able to develop new products at a rapid pace. Because of their highly developed research capabilities some of the companies specialising in supporting the research sector may be able to launch services related to the screening and modification of biocatalysts for various sectors of industry.

The specification, discovery, and modification of enzymes for industrial or pharmaceutical applications are time- and resource-consuming tasks, and there is a need for technologies that would further automate and quicken searches, screening tests, and modification procedures. It can be expected that methods will be developed to make direct DNA screening of enzymes more feasible. Similarly it can be expected that methods of rational modification will become more important for well-characterised enzyme-substrate pairs.

Services related to biocatalyst technologies are required by customers from all segments of the biocatalyst user community. Yet there are wide differences between the frequency and quality of services required by bulk commodity producers, fine chemicals manufacturers and research laboratories. While industry may need support mainly in process design, research laboratories can require detailed information on even the most improbable aspects of the products they use. Industry is likely to seek advice from third parties for economic reasons, but in research settings users want to be reassured about the characteristics of the products in absolute detail, and it is the manufacturers of enzymes who are expected to supply all of the required information. Producers of fine chemicals have to be able to combine various areas of expertise and may be willing to use consultant services relatively frequently.

2.3.6 The influence of user requirements on biocatalyst technologies

Methods have to be developed to modify the properties of biocatalyst technologies so that they will meet future requirements of industry. Many of the natural enzymes have to be made more efficient and stable in wider temperature ranges, at high pressures, or even in non-aqueous environments, such as organic solvents.

Methods used to modify existing (natural or man-made) biocatalysts include standard chemistry, recombination techniques (standard cloning, expression cloning, DNA shuffling), rational design methods (usually involving computerised methods and databases) and directed evolution (multiple cycles of mutation, selection, and amplification). With modification some enzymes can be made resistant to inhibitors that usually restrict their use. Finally, enzymes intended for the marketplace have to be supplemented with bulking, coating, and preservative compounds.

Improved enzyme stability properties can be achieved by crystallising and crosslinking the enzyme protein, one example being glucose isomerase (Visuri 1992). The resulting immobilised enzyme retains most of its activity and can be filtered for reuse allowing cost savings. Some cross-linked enzyme crystals withstand exposure to high temperatures, extremes of pH, and various solvents. In industrial applications immobilisation of an enzyme on a solid support makes it possible to recover the catalyst by filtration, but immobilisation reduces the volumetric activity of the catalyst by 50-to-100 fold. Often the advantages of successful immobilisation outweigh the disadvantages. In antibiotic production immobilised penicillin acylace can be reused over 1 000 times. (Lalonde 1997.)

A complementary approach is to use enzymes from micro-organisms and other life forms discovered in extreme habitats. Thermophile micro-organisms have been found in hot springs and in the neighbourhoods of deep sea hydrotermal vents, where the temperature and pressure are extremely high. Archaebacterium *Pyrolobus fumarii* thrives within the temperature range of 95 °C and 113 °C, but the pasteurisation temperature of 85 °C is too low for its growth (Blöchl *et al*. 1997: 19). Known hyperthermophiles include both proteolytic and saccharolytic heterotrophs, methanogens, sulfur-respiring autotrophs and heterotrophs, microaerophiles, sulfate reducers, iron oxidisers, and nitrate reducers (Adams and Kelly 1998: 329). These organisms rely on enzymes that are efficient in conditions that resemble those prevailing in the process and chemical industries. Moreover, thermophiles have enzymes that can degrade interesting sulphur and metal compounds.

The search for thermostable enzymes is facilitated by the fact that the full genome sequences of extremophiles are becoming available. The first results indicate that the protein structures of thermostable enzymes differ surprisingly little from those of conventional enzymes (Adams and Kelly *ibid*: 330). If this finding is sustained, it adds to the interest of research on enzyme modification, as relatively small changes in the molecular structures of enzymes may change their properties dramatically. Enzymes retaining their activity in cold temperatures can be found in species living, for instance, in the seas of the Antarctic, where the body temperatures of the fish may be as low as -1,8 °C. Enzymes from organisms adapted to cold conditions can be useful in the food industry, waste treatment and environmental remediation. (Russell 1998.)

2.4 Conclusions

Biocatalysts are catalysts of natural origin stimulating or precipitating chemical reactions in living organisms or industrial processes. In the short run research is likely to identify increasing numbers of enzymes that are useful in the degradation and synthesis of important industrial, medical and environmental compounds. At the same time progress is going to be made in characterising factors that influence conditions in which particular enzymes can be applied. Taking advantage of the purity of processes catalysed by enzymes is an important consideration expanding their application. Yet it takes time to redesign industrial processes so that the most can be made of the beneficial properties of biocatalysts. That is why the range of uses of industrial enzymes grows only at a modest pace in the short run.

In the medium run the best commercial prospects can be found for biocatalysts in the production of fine chemicals that can sustain the relatively high economic costs of biocatalytic processes. New products will emerge from enzymatic processes that take advantage of the wealth of intermediate compounds created in industries processing bio-originated materials. Biocatalytic processes of the future may turn common bio-originated materials into highly-priced intermediates of fine chemicals, valuable pharmaceuticals, or ingredients of functional foods.

In the medium run there is also room for new businesses specialising in biocatalyst technology. New enzyme manufacturers serving research and laboratory requirements are likely to emerge. There is also need for new service companies specialising in advanced industrial process development and design. Services

related to screening and modification of new biocatalysts is another promising area for companies that have acquired high expertise in biocatalyst technology.

In the long run it is possible that a new holistic approach emerges in enzyme biotechnology. Such an approach could focus on rapid development of new enzymes and enzymatic processes for particular needs emerging in industry. Elements of that approach could include the use of molecular and DNA databases in screening for enzymes, highly automated combinatorial methods in the production of new enzyme compounds, powerful computerised molecular modelling systems in designing new or hybrid enzymes for particular purposes, and versatile microreactor systems in testing process designs.

3 Biomaterials

3.1 Defining three types of biomaterials

There are three main types of biomaterials: bio-originated, biocompatible, and biodegradable materials. Bio-originated materials are produced by biological organisms, and it is their origin that makes these compounds biomaterials. Silk, wool, wood, and cellulose are biomaterials of this type. Medical and veterinary biocompatible materials are used to support, enhance, and even replace natural organs. In this case it is the application domain that makes even a synthetically produced compound a biomaterial. Medical biocompatible materials include those that are used in surgical instruments, prostheses, and implants.

Bio-originated and biocompatible materials are useful because of their structural rather than chemical properties. In contrast the defining feature of biodegradable materials is that their chemical composition is subject to rapid decay as a result of biological action. In surgery biodegradable materials are often called biosorbable materials; after having fulfilled their purpose they have the great advantage of not requiring a secondary operation for their removal. Ecologically biodegradable materials are relatively easy to dispose of after use as they are designed to decompose rapidly when exposed to light or aerobic or anaerobic processes. For these materials there are natural processes that treat them as valuable inputs rather than waste.

3.2 Biomaterials of today

3.2.1 Bio-originated materials of today

Bio-originated materials are usually also renewable raw materials and ecologically degradable. Carbon dioxide is an example of a compound that is released at a rate at which it cannot be fully utilised by natural processes and instead accumulates in the atmosphere to an extent that causes concern. Highly efficient technologies of a distant future could turn carbon dioxide into a feedstock for the chemical industry. It is abundant, inexpensive, and not flammable. Recently advances have been reported in efforts to develop catalysts for the alternating copolymerisation of carbon dioxide with epoxides to produce aliphatic polycarbonates (Cheng *et al*. 1998). Biocatalysts play a key part in natural processes involving bio-originated and biodegradable

materials; indeed many bio-originated materials can be modified by using biocatalytic reactions.

In contrast to non-renewable raw materials exploited by extractive industries most bio-originated materials are composed of elaborate chemical and physical constituents. In industrial applications the anisotropic structure of the natural materials is usually broken down, and the most useful compounds are selected for purification. For some natural materials the structure of the material is more important than its constituent mass. In pulp production wood material is broken down and ground or boiled into fibres. It is the branched and tangled mesh structure formed by cellulose fibres rather than the weight of its mass or its chemical properties that is exploited in paper production. Ultimately the properties of bio-originated materials are determined by the DNA sequences and gene regulation mechanisms of the producer organisms; accordingly ways may be found in the future to modify at least some of the important bio-originated materials of today.

The most important traditional bio-originated raw materials include polysaccharides such as starch (in food and beverages), cellulose (in timber, paper and cotton); proteins including keratin (in wool), fibroin (in silk), and collagen (in leather); lipids and lipoids (in industries using fats and oils). Of all bio-originated materials cellulose is the most abundant. Cellulose can be hydrolysed to form glucose that in turn can be converted into ethanol and fine chemicals. Similarly hemicellulose can be turned into xylose or glucose, and starch can be converted to alcohol and a number of other products. It looks possible to develop bacteria that are able to produce ethanol from cellulose, although the cost will be a problem for a considerable length of time. So far a strain of Gram negative enteric bacteria *Klebsiella oxytoca* has been engineered to produce ethanol from cellulose, but researchers aim at being able to use Gram-positive bacteria in the future and to further develop enzymatic processes in order to eliminate some pretreatment stages. (Ingram *et al*. 1998.)

Materials that have been modified or produced by biotechnical means sometimes find new uses in non-traditional fields. Alginate, a structural polysaccharide used by the food industry as a gelling agent, can be applied to encapsulate animal cells in immunoisolated transplants, in which for instance Langerhans islets thrive and produce insulin for months (Bucke 1998). When large quantities of a biomaterial are not required and when price is not the main consideration, traditional biomaterials may be replaced with purer forms produced by microbes. Cellulose can be produced

by *Acetobacter xylinum* and other bacterial species; bacterial cellulose is a highly priced speciality product used in wound dressings, temporary skin substitutes and in acoustic-diaphragm membranes of personal stereo devices (Sutherland 1998: 41-42).

3.2.2 Medical biocompatible materials of today

Medical biocompatible materials of today are so valuable that up-to-date technologies can be economically applied in their production; in the future such technologies may include methods that include molecular manipulation techniques of nanotechnology. At present medical biocompatible materials include polymers, metals, ceramics, and composites. Medical biocompatible materials are required only in small amounts; sometimes it is the short production runs that have contributed to quality problems in plastics used in artificial joints, for instance.

An important determinant of the biocompatibility requirements of a material is the designated duration of its contact with living tissues. Some materials are intended to be used in diagnostic instruments or during surgical operations and others during long periods of convalescence, while some are to remain implanted permanently. Patient monitoring equipment as well as kidney dialysis and heart-lung machines remain in body contact for extended periods of time, and their materials face body chemicals and stresses similar to those met by artificial implants, but unlike implants such devices are used in hospital conditions.

Most of the classical materials applied in medical sciences, such as steel, titanium and aluminium, are intended to remain chemically *inert* when in contact with the human body or, if implanted, to be capsulated harmlessly by fibrous tissue in the body. Some other materials, including certain forms of bioglass and ceramics, are *bioactive*. They allow or even promote the growth of tissues they are in contact with, and some of these materials form strong bonds with bone, enabling their use in fixing artificial joints. *Resorbable* biomaterials, such as tricalcium phosphate and polylactic-polyglycolic acid copolymers are useful in orthopaedic surgery because they do not have to be removed surgically, and the need for a secondary operation is avoided. Table 3.1 lists some medical applications of biomaterials.

Table 3.1. Some types of available or experimental medical biocompatible materials and their applications.

Interfacing tissue	Chemical properties of the biomaterial		
	Inert	**Bioactive**	**Degradable**
Bone	Metals, ceramics	Bioglass	Lactic acid derivatives
Dental and gum tissues	Mercury alloys, polymers	Atrisorb (regeneration of gum tissue)	Degradable surgical materials
Cartilage	Bovine collagens	Regenerated human collagen	Degradable surgical materials
Skin	-	Engineered dermic and epidermic skin transplants	Temporary skin substitutes for burn and wound dressings
Blood	-	Fluorocarbon emulsions	Fluorocarbon emulsions
Intestinal tract	Polymers	-	-
Kidneys and urethra	Laboratory-grown cartilage tissue	-	-
Heart	Polymer materials for heart valves	-	-
Lung	-	-	-
Sense organs (eyes, ears)	Cochlear implants	Artificial bioactive cornea transplants	-
Peripheral nervous system	-	Bioactive materials furthering the growth of aksons	-
Central nervous system	-	Bioactive materials furthering the growth of dendrites	-

Source: Business Week July 27th, 1998.

Most implants of today are used to supplement or replace lost or weakened structures in the body; artificial implants restoring or augmenting other than mechanical organ functions are still relatively rare. Materials used for filling tooth cavities as well as dental, orthopaedic, and plastic surgery implants are the most common applications. Their materials are intended to strengthen tissue structures of the body and to remain chemically inert. However, some psychological discomfort may be involved with their use, and if the legal system provides suitable incentives, even materials well known and widely used for decades may suddenly become subjects of public controversy and class-action suits, as demonstrated by dental

amalgam (Ekstrand *et al*. 1998) and silicone-implant cases (Diamond *et al*. 1998). In the future litigation risks may raise further the costs of developing and manufacturing medical biocompatible materials.

Although artificial joint implants are manufactured from the best available materials, their long-term durability remains a problem. Artificial joints consist of a plastic cup, made of high molecular weight polyethylene; inside the cup there is a ball affixed to a metal stem. The ball is made of metal (titanium or cobalt chromium alloy) or ceramic (aluminium oxide or zirconium oxide). Artificial joints often fail in 10 to 15 years. The grinding movement of the joint releases minute amounts of polyethylene that tend to adhere to the ball. Submicrometer-sized wear debris is also released and leads to an immune system attack that may cause osteolysis, the death of bone cells adjacent to the implant. Over time the process leads to bone being resorbed from around the implant which then gets loosened. (Sloten *et al*. 1998: 1455.) Finally the joint may have to be replaced. The search for stronger materials and better designs continues.

3.2.3 Ecologically degradable materials of today

In packaging materials, hygiene products as well as in disposable dishes and cutlery, environmentally friendly biodegradability is an important competitive factor. Demand for degradable materials is driven by environmental concerns, government regulations and public image considerations. In the European Union the directive on packaging and packaging waste (94/62/EC), adopted in 1994, sets the target for the proportion of packaging waste that is to be recovered at between 50 % and 65 %. Between 25 % and 45 % of the totality of packaging materials is to be recycled; for each individual packaging material the minimum recycling target is set at 15 %.

Technical definitions and test procedures related to biodegradability issues have been defined by the European Committee for Standardisation (CEN) and the American Society for Testing and Materials (ASTM). Biodegradation has been defined as degradation caused by biological activity, especially by enzymatic action, while biodegradable plastic is defined to be a plastic that can be degraded by naturally prevalent micro-organisms. (Riggle 1998.)

While definitions, testing procedures and standards help to establish regulatory frameworks for efficient markets of biodegradable materials, such markets can be expected to remain niche segments. In the near future the market for environmentally biodegradable plastics is estimated to be at most one thousandth of production quantities of traditional plastic compounds (Lubove 1999). The cost of producing biodegradable plastic through microbial fermentation has been estimated to be about ten times as much as making plastic from petroleum (Kilman 1999). That is why many of the large international producers will not be seriously interested in these products. Some of the large companies have quietly abandoned biodegradable products, and there may be opportunities for small producers if they are able to establish a special status for their products and cover the high costs of small production runs by charging relatively high prices.

3.3 Technological opportunities in biomaterials

Biotechnology offers several ways to improve the properties of bio-originated materials. The methods range from modifying the DNA of producer organisms to enzymatic treatments and biodegradation of waste materials. The efficiency of production may be increased by transferring genes expressing the biomaterial proteins into microorganisms or plants. In the future cotton may be modified to produce fibres of different colour, water resistance, and strength. Corresponding advances may be possible in modifying cellulose materials produced in trees.

3.3.1 Foreseeable trends in medical biocompatible materials

In the past the basic idea of surgery was the identification and *removal* of damaged or diseased tissue. At present biocompatible materials and tissue transplants are used to *replace* such tissues. Over one million people have benefited from organ transplants, and both in Western Europe and in the United States tens of thousands of people are waiting for transplant kidneys. (Rochi 1998: 23.) In the future it may become possible to apply tissue *regeneration* methods in supplying spare parts for humans (Hench 1998: 1420).

One of the promising medical biocompatible materials is bioactive bioglass. In the future it may become possible to develop bioglass that is not prone to forming

cracks and thus would be strong enough for load-bearing applications such as artificial joints. Bioactive glass could be designed to release proteins and growth factors that promote the growth of bone and soft tissues. At the same time biocompatible materials may be engineered to prevent the growth of bacterial biofilms on the surfaces of artificial implants. Biofilms are difficult to eradicate, because their slime matrix protects the bacteria from antibiotics. Endotracheal tubes, intravenous catheters, urinary catheters, heart valves, dental implants, and even joint replacements are sometimes affected by biofilms and have to be removed. One way to prevent the growth of biofilms is to apply thin silver coatings on implants by using ion-beam assisted deposition (Blanchard 1995). Another is to prevent bacterial communication that triggers the formation of biofilms at least in some cases (Davies *et al*. 1998).

Shape-memory metal alloys have properties that seem to be useful in surgical applications. When the temperature of a memory metal rises above a certain treshold level, the metal returns to a predesigned shape. A metal implant made of nickel-titanium alloy (NiTi), for instance, can be made to expand or contract to a predetermined shape at the body temperature. The alloy seems to have a particular promise in self-expanding stents that can sometimes be used to replace the need for a major surgical operation. (Ryhänen 1999: 53.) In biocompatibility tests the material has shown good potential for clinical use (Ryhänen *ibid*; Wever *et al*. 1998).

Special fluorocarbon emulsions can be used temporarily as artificial blood to augment supply of oxygen in patients who are suffering from tissue hypoxia or ischemia. Artificial blood substitutes could be particularly useful in trauma situations, and they might save costs of donor blood processing. (Riess and Krafft 1998: 1529, 1536.) Human, bovine, and genetically engineered hemoglobin may also offer medical opportunities. *Escheria coli* bacteria have been engineered to produce modified hemoglobin that carries oxygen efficiently (Weickert and Apostol 1998).

Fundamental problems related to the interface with blood remain to be solved in efforts to develop mechanical heart replacements. Several left ventricular assist devices have been developed (NIH 1998: 9), but their materials tend to lead to blood clotting and strokes. At present heart-lung transplants have a better record of success than mechanical heart replacements.

An important goal in the development of medical biocompatible materials is to reduce their tendency to activate human immune defence systems when placed in permanent contact with living tissues. Promising materials include polyethylene oxide, which resists protein pickup, and hydroxyapatite, which is said to assist healing without being capsulated in the process. (NIH *ibid*: 6.) An ideal solution is to use autografs or cells that originate from the patient and have been cultivated to the required quantity and shape. Cartilage cells removed from the patient can be implanted into a joint to repair small lesions. In a new treatment a meniscus is replaced by a collagen scaffold that encourages the regeneration of the patient's cartilage tissue.

A complementary approach would be to find ways to modify the responses of the human immune system so that selected foreign materials and tissues would be treated as normal body parts. An improved ability to modify immune responses selectively could unfold new medical technologies that would improve the success rate of surgical implants and might also be used to alleviate allergies, asthma, rheumatic conditions, diabetes, psoriasis and multiple sclerosis. CD4 T cells play a crucial role in the rejection response of organ transplants, and their activity is heightened by secondary chemical signals received after the cells have recognised the transplant antigen. That is why the immune response can be modulated to some extent by the inhibition of the secondary signals. As a result of the inhibition therapy, harmful CD T cells may lose interest in the transplant, fail to secrete cytokines to alarm other parts of the immune system, and eventually undergo programmed cell death. (Denton *et al*. 1999.)

Tissue regeneration is based on tissue engineering, which involves cultivating cells of human organs artificially *in vitro* or *in vivo*. In the future these technologies may result in the development of methods that can be used to produce viable organs, and consequently the need for donor organs may be reduced. Tissue engineering could also help to avoid the risks associated with the use xenotransplants. As diseases that have crossed from animals to humans include AIDS, BSE-CJD, and some types of influenza, the health risks of xenotransplants may be considerable.

When human cells supplied with nutrients and growth factors are cultivated on a three-dimensional scaffolding consisting of polymer compounds, the cells reassemble into structures that resemble the original tissue. The challenge for biomaterial engineering is to develop matrix materials that support and possibly accelerate the growth of human cells. (Kim and Mooney 1998.) Another important

task is to develop materials for capsules that can sustain *in vivo* for extended periods cells secreting vital proteins such as insulin. Such polymer capsules should allow the passage of small molecules (nutrients and therapeutic molecules) but prevent the flow of large molecules, such as those from the immune system. Cells protected by capsules could remain alive and unrecognised by the immune system. If successful, tissue cultivation might result in transplant tissue banks serving large populations. A critical factor will be the extent to which it will be possible to control human immune responses in the future. An ideal solution would be to develop methods that can be used to cultivate patients' own cells on demand.

The ability to use of human stem cells would greatly increase the potential of tissue engineering techniques. At present undifferentiated human stem cells can be obtained from a fertilised human egg after the first few rounds of cell division. Blood-forming hematopoietic stem cells can be obtained from adults and are used in bone marrow transplants. The recent discovery of adult mesenchymal stem cells that can differentiate into bone, cartilage and fat tissue demonstrates the potential of this field of research (Pittenger *et al*. 1999). In the future stem cells obtained from a patient or artificial stem cells prepared by using the patient's DNA might be applied in transplants and directed to grow to become the required tissue, for instance the insulin producing beta cells of the islets of Langerhans.

Human skin is a promising starting point for tissue engineering, as the body's immune system does not usually reject dermic allografs or even artificial dermic substitutes. Human skin products have already received the Food and Drug Administration (FDA) approval in the United States. One piece of a baby's foreskin can be used to produce more than 1.5 hectares of engineered skin having a five-day shelf-life. One product has been described as behaving clinically like an autograft and being morphologically, biochemically, and metabolically similar to human skin. (Kirsner *et al*. 1998: 248). Other organs on which cultivation research is being carried out include cartilage, blood vessels, pancreas, and even liver. (Arnst 1998: 58.)

The cultivation of neurons will be a special challenge. Until recently it was thought that neurons (with the exception of olfactory receptor cells) do not replicate in adults. Then neuron generation was discovered in adult rats, marmoset monkeys, and some birds. In November 1998 convincing evidence was presented on the regeneration of mature neurons in the adult human hippocampus, a centre of memory and learning in the brain (Eriksson *et al*. 1998). In the future it may become

possible to implant neurons and stimulate their growth in the brain and elsewhere in the body.

Under the right conditions axon extensions of the peripheral nervous system can be induced to grow and re-establish functional contacts across wounds caused by injury. Long gaps can be healed in the future by implanting grafts acting as bridging or guidance channel constructs. Collagen is a promising material for such grafts. Resorbable materials of future grafts may be engineered to release nerve growth factors to stimulate the healing process. Many of the neurotropic factors required are released by Schwann cells, which play an important role in natural nerve regeration. That is why the presence of these cells may be desirable in future nerve grafts. Schwann cells have been found to enhance the regeneration of nerve cells both in the periferal nervous system and in the spine. (Heath and Rutkowski 1998.)

Interface materials connecting living tissues with artificial devices are increasingly required in many areas of advanced medical technology. Over the last few years it has become possible to restore some hearing to deaf patients by using cochlear implants to relay microphone-generated signals to fibres of the hearing nerve. The functionality of these implants has been improved by increasing the number of electrodes used to stimulate the hearing nerve and by including speech decoders. Even so, sense organs remain a challenging area for artificial implants. Some progress has been achieved in developing artificial cornea implants consisting of materials that permit colonisation by host keratocytes. Colonisation integrates the materials into the recipient cornea. (Legeais and Renard 1998: 1517.)

Materials that are used in connecting artificial devices to nerve fibres must have suitable electrical properties. Recently special pacemakers stimulating the brain via the *vagus* nerve in the neck have been used to ameliorate epileptic seizures. (University of Maryland 1997.) New types of pacemakers can be expected to be developed to treat some of the conditions that involve irregularities of the heart beat. It is likely that the use of microelectronic processors will gradually increase in applications involving the peripheral and the central nervous system. (Heiduschka and Thanos 1998.)

A computer connected directly to a brain implant has already given a seriously disabled person some ability to communicate with the outside world. Two hollow glass cones about the size of the tip of a ballpoint pen were placed in the most active region of the motor cortex of the patient. The glass cones, laced with neurotrophic

chemicals extracted from the patient's knee, encouraged nerve growth. Within several months neurons in the cortex grew pathways into the cones and attached themselves to electrodes mounted inside. As the patient was asked to think about moving various parts of her body, the responses of the electrodes were monitored and the response patterns were translated into commands for the computer cursor. Now the patient can move the cursor by thinking body movements and thus slowly communicate with the outside world. (Graham-Rowe 1998; Helsingin Sanomat 1998.)

3.3.2 Foreseeable trends in bio-originated materials

In the future energy production will continue to be one of the main uses of bio-originated materials. Methane can be obtained from anaerobic biogas digesters that are fed by putrescible municipal solid waste and sewage sludge (Rintala and Järvinen 1996) and remains a useful source of energy and chemicals. Hydrogen can be produced by a cyanobacterium mutant that lacks uptake hydrogenase activity. The bacterium converts light into hydrogen at an efficiency of 1.4 %. (Markov S.A. 1997.) In energy production biotechnological methods may be used also to improve fuel properties. Microbes for the desulfurisation of coal (Rubiera 1997) and flue gases (Grootaerd 1997) are already being tested.

Many bio-originated materials can be treated by using naturally evolved enzymes, but their relatively high cost often precludes their use in the production processes of bulk materials. Possible applications of enzymes include the treatment of cellulose and hemicellulose in pulp, fabrics, and bread. Cellulases can give cotton a silky look and feel. Enzymes can also be used to remove impurities which are a problem for most bio-originated materials. In the case of cotton, impurities include lipids, waxes, and pectins. (Tenkanen *et al*. 1998.) Thermostable enzymes are often more productive than their conventional counterparts. Xylose isomerases from *Thermotoga* are stable at temperatures over 80 ºC and produce higher amounts of fructose from glugoce than enzymes that operate in mild temperatures. (Adams and Kelly 1998: 331.)

Proteins from organisms adapted to cold habitats may find applications in food and medical technologies. Tissues and blood serum of cold-water ocean fish contain antifreeze proteins that inhibit the growth of ice crystals. If the cost of these proteins

could be significantly reduced, they could be used in food technology to preserve the properties of frozen foods. In the future genes expressing such proteins maybe could improve frost resistance of modified agricultural plants and vegetables (Freeney and Yeh 1998: 105). The antifreeze proteins may be used also to protect transplant organs during transport, and they could be useful in surgical operations carried out at very low body temperatures.

Gene technologies can be expected to have a great impact on the efficiency at which bio-originated materials are produced. Genetically modified cotton will not only grow faster but it can also incorporate insecticides. In modified tree species lignin may be removed from cellulose more easily than at present, saving energy in pulp and paper production. Enhanced insect and herbiside resistance may be also introduced into cultivated tree species. Plantations of modified trees may be used in the future to remove heavy metals and other toxic substances from the soil. (Tzfira *et al*. 1998: 441-442, 445.)

Bio-originated polymers are another interesting research topic. Over the past decade it has been proven that bacteria are capable of synthesising a wide range of aliphatic polyesters that can be used as raw materials in the production of biodegradable thermoplastics and elastomers. At present, aliphatic polyesters can be produced by microbial fermentation. In the future their production may be carried out in vitro or in agriculture by raising suitably modified transgenic plants. In a recent experiment, a plant was modified to produce a polyester compound. The result was that 14 % of the plant's dry weight consisted of the compound. (Steinbüchel and Füchtenbusch 1998: 424.)

Bacteriorhodopsin is one of several bio-originated materials that have raised interest in electronics and optoelectronics. The protein is found arranged in sheets in the cell membrane of *Halobacterium salinarium (halobium)* and consists of about 4 000 atoms. Bacteriorhodopsin absorbs photons of visible light and is used by the bacterium to generate energy in the form of ATP molecules. Bacteriorhodopsin harvests light by using a molecule that can also be found within the rod cells of vertebrate eyes. In the future it may become possible to immobilise the protein in liquid crystal components and other electronic devices. (Beckmann *et al*. 1997.) The photon-induced reactions of the rhodopsin proteins are sensitive, energy efficient and rapid, and in the future they may be exploited also in photodetectors and in display devices.

Judicious use of biomaterials as prefabricated components in nanotechnology could speed up the development of that field and give birth to new electronic, sensor and diagnostic devices. Molecular biology offers an abundant supply of structures and mechanisms of nanometer scale. Rapid computerised means to locate useful components could be developed, and compared to manipulations of individual molecules the exploitation of natural-made components would probably be much more efficient and make useful applications feasible. It may also become possible to build nanobiological devices that incorporate functional biological components. In such uses special care would have to taken to retain the functionality of the biological molecules. Interesting bio-originated mechanisms discovered so far include rotary action in ATP synthase, in which chemical energy is converted to mechanical energy at an efficiency of almost 100 %. Another efficient rotary nanomechanism is the flagellant motor that propels the movement of some bacteria. (Berg 1998; Oplatka 1998.)

Potential sources of nanotechnical mechanical energy include peptide-based polymers that are able to convert heat as well as chemical and electrical energy to mechanical movement by contracting or stretching. The motion relies on the difference between an ordered and disordered state that is mediated by the relationship of water molecules to hydrophobic molecules in the polymers. Changes in temperature, pH, or pressure can trigger a transition that results in a muscle-like contraction or stretching. In the future it may become possible to exploit these phenomena in artificial implants that mimic the elastic properties of human tissues. (Urry 1995, 1998.)

3.3.3 Foreseeable trends in ecologically degradable materials

The first generation of degradable polymer products was made to decompose by adding starch between polyethylene polymer chains or by including compounds that would degrade as a result of exposure to light, heat or water. Polyethyle itself was and remains unbiodegradable. The technology can be made more sophisticated by using additives that control the onset and pace of the decomposition process.

Full biodegradability has been achieved in polymer products of the second generation. Designed for complete degradation in microbial environment, these materials include aliphatic polyesters, polylactic acid polymers, and microbial

polyesters (Riggle 1998). Among microbial polyesters polyhydroxyalkanoates (PHAs) and their variants PHB and PHV have received a great deal of attention in recent years. The advantages of PHAs include biodegradability, medical biocompatibility, and modifiability. The properties of PHAs resemble those of petrochemical thermoplastics. The compounds range from being rigid and brittle to flexible elastomers. Moreover, PHAs can be converted into intermediates of valuable fine chemicals. The economics of microbial PHA production can be somewhat improved in the future. It is possible to raise the productivity of the microbes, increase the PHA content in the microbial dry cell weight (the record being 88 %), and develop improved recovery methods. (Choi and Lee 1999: 19.)

The scope of materials considered to be biodegradable is likely to grow in the future. Almost all materials are subject to biodegradation, if sufficient time can be allowed, and even highly poisonous substances may be treated biologically so as to remove some of the most toxic components. Certain anaerobic microbes are able to dechlorinate organochlorine compounds such PCBs, DDT, and chlorinated aliphatics, such as the industrial solvent trichloroethylene (TCE). However the degradation processes are slow, in practice often incomplete, and produce toxic compounds (Bonaventura and Johnson 1997). Some bacterial strains are resistant to metals such as mercury and cadmium, for instance (Ramanathan *et al*. 1997: 502). One particular microbe species thrives in highly radioactive conditions and perhaps can be modified to digest radioactive waste materials (Lange *et al*. 1998).

There are microbes, particularly fungi, which can attack some forms of glass. Therefore antimicrobial coatings have been applied on glass since the 1940s (Drewello and Weissmann 1996: 339). Some bacteria, such as *Thiobacillus ferrooxidans*, derive energy by breaking down metallic sulfides and releasing free metals including copper, gold, lead, and zinc as well as sulphur acid (Bonaventura and Johnson 1997). Bacterial leaching processes are applied to recover metals from low-grade ore, fly ash and industrial wastes. It has been estimated that more than 10 % of world copper, gold and uranium production is based on bacterial leaching (Brombacher *et al*. 1997: 578). The methods applied include bacterial leaching *in situ*, ore heaps, series of vats, and reactors consisting of stirred tanks.

In order to be successful, a company producing ecologically degradable materials has to be able to command relatively highly prices. This might become possible by developing a product line of biodegradable additives that enhance the properties of bio-originated materials such as paper, cotton, or wood. The most promising

applications of biodegradable materials may therefore be found in inks, dyes, paints, coatings and in chemicals used to treat bio-originated materials. In some cases these applications may be of such value that microbial processes can be developed for the production of the biodegradable additive. It may even become possible to transfer the relevant microbial genes into the organisms that produce the bio-originated materials. Cotton fibre of the future may contain ecologically degradable polymers in its hollow center, lumen; genetically engineered trees of the future could have their fibres strengthened by biopolymers.

One possible application of ecologically degradable materials is their use in enhancing the properties of recycled bio-originated materials. Biodegradable polymers could be used to strengthen recycled paper so that, for instance, semi-durable boxes and containers for home and office use could be fabricated.

3.4 Conclusions

There are marked differences in the future prospects of the three main types of biomaterials: bio-originated, biodegradable and biocompatible materials.

In the short run technical opportunities in bio-originated and ecologically biodegradable materials are rather limited. The properties of some of these materials can be slightly modified, but probably not to an extent that will significantly change their positions in the marketplace. Even in the long run there are few encouraging signs of progress in ecologically biodegradable materials. They have to compete with traditional bio-originated products such as paper and wood that have adequate biodegradation and recycling properties for most purposes. Another factor limiting prospects in this sector is the lack of real evidence for the willingness of customers to pay for ecological biodegradation properties. That is why it is reasonable to expect that even in the medium and long run the best prospects for ecologically biodegradable products lie in supporting traditional materials such as paper, wood and various textiles. Forecasters usually tend to be more upbeat than this in evaluating the prospects of ecologically biodegradable products.

In medical biocompatible materials the situation seems to be much more lively even in the short run, and it is likely that new applications will be developed for these materials at a rapid pace. In the medium run the prospects are even brighter, as the application of the methods of the present biotechnical paradigm will most likely

produce significant improvements in the interfaces between biocompatible materials and living tissues. Gradual advances, but not complete success, can probably be achieved in tempering the response of the immune system against at least individual biocompatible materials. When combined with expected progress in materials themselves, it seems that many of the problems that concern the use of artificial joints, for instance, are likely to be solved in the medium run. Biomaterials that are used to replace structural body parts will become increasingly reliable.

In the long run the most significant change involving the use of medical biocompatible materials is likely to be the adoption of tissue engineering technology. In the long run it will become possible to cultivate parts of some organs in vitro and use them as surgical implants. Medical biocompatible materials are likely to play a significant role in tissue cultivation, protect implanted tissues in the body, and enhance their growth. It will take decades of determined work before the required social and technological infrastructures will be built to the extent that tissue engineering methods will have any significance in national health statistics. The social infrastructures required include appropriate regulations and the creation as well as dissemination of adequate skills. Technological infrastructures needed include tissue engineering platforms, transport arrangements, and tissue databases. The present generation of adults is not likely to profit from these technologies.

In the long run the properties of many bio-originated materials are likely to be modified extensively by using methods of genetic engineering. The limiting factors concern both the technical difficulties involved and the uncertainties about ecological safety, regulation, and public attitudes.

4 Medical diagnostic technology

4.1 Defining medical diagnostic technology

Medical diagnostic technology can be defined as techniques and methods used in the detection and quantification of physiological and medical indicators of human health. Differentiation between biotechnology-based and other forms of diagnostics is somewhat arbitrary. The defining feature of biodiagnostics is the use of biological materials in sensor or reagent components. The borderline is blurred by modified enzymes and compounds that have bio-originated and synthetic constituents.

Medical diagnostic technology is distinguished by the wide range of its constituent technologies. Medical diagnostic systems are based on combinations of several technologies that are used in the handling of test samples, pretreatments, information acquisition from samples as well as in processing, distribution and storage of the acquired information. Rapid advances are expected in many of the constituent fields of medical diagnostics, and that is why the field as a whole can be relied to develop at a rapid pace in the future. Moreover, companies developing technologies for medical diagnostics are aware that some of the techniques have potential also in monitoring environmental or industrial processes. As the importance of information processing increases in diagnostic systems that are becoming more integrated and automated, so does the value of software and networking standards.

Our focus is on new technological opportunities that are opening up in medical diagnostics. Attention will be paid to sensor components of diagnostic systems software, data networking, automation, image analysis, and miniaturised systems. The discussion relates mainly to clinical laboratory tests and *in vitro* diagnostics, but an attempt is to made to recognise the potential of *in vivo* diagnostic technologies as well.

4.2 Medical diagnostic technology today

Established diagnostic technologies are based on reagents, such as enzymes, which indicate the existence of a particular substance in the studied sample. In some applications enzymes have been replaced with very specific and reactive antibodies. The changes in the reagents are measured by using luminescence, fluorescence,

light absorbance or radioactive techniques. Immunological assays, including radio-immuneassays (RIA) or enzyme immunoassays (EIA, ELISA) are used to diagnose bacterial infections and to determine the levels of hormones, growth factors, and other substances.

Although invented already more than 20 years ago, the use of monoclonal antibodies is still widening in diagnostics. Antibody-based tests are rapid, producing results in minutes, and highly sensitive, sometimes requiring only a drop of blood. Often these tests are simple to administer and interpret and are therefore at least in principle suitable for point-of-care use. In in-vivo diagnostics antibodies can be used to carry various label substances to specific targets, such as cancer cells, which can then be detected by using X-rays or other means.

The wide variety of diagnostic tests is usually divided into four categories: chemistry, diagnostic immunology, microbiology and haematology tests. A fifth category, tests related to the human genome is expected to expand rapidly in the future. Similarly the number of available microbial tests will grow as a result of DNA tests of pathogen genomes. Table 4.1 presents a classification of diagnostic tests and some of the foreseeable development prospects for these types of tests. The classification is based on U.S. regulations presented in Clinical Laboratory Improvement Amendments (CLIA) regulations (Schwartz 1999: 740).

Table 4.1. A classification of diagnostic test types and some of their development prospects.

Target	Short explanation	Future developments
Microbiology	Tests related to bacteria, viruses, and parasites.	DNA-chips are expected to yield rapid and accurate results.
Diagnostic immunology	Tests related to the immune system, including lymphocytes, monocytes and immunoglobulins.	DNA-chips will enable the determination of genetic features of individuals' immune systems.
Diagnostic chemistry	Includes endocrinology and toxicology tests.	Increasingly accurate and automated tests.
Haematology	Including coagulation, blood profiling, and immunohaematology tests.	New miniaturised point-of-care devices.
Pathology	Including histopathology and neuropathology tests.	Application of DNA-based assays.
Cytology	Including cytogenetics tests on genetic factors affecting cells	Application of DNA-based assays.
Histo-compatibility	Compatibility tests for tissue transplants	Traditional serological tests will be replaced by DNA-based tests.
Genetics	Tests for genetic defects and polymorphisms.	Acquisition of genetic information on individuals by using DNA chip.

In terms of world wide sales, microbiology and immunodiagnostics tests constitute the largest markets. Microbiology tests are carried out to detect and identify bacteria, viruses, and parasites. Bacteria are the most common targets. Among the relatively few tests to diagnose virus diseases are HIV and hepatitis C tests. Most of the bacteriology tests of today require cultivation of samples, which slows down the process and introduces a potential source of error. Challenges facing the field include the need to develop methods for rapid identification of bacterial strains that have developed antibiotic resistance. The HIV, chlamydia, and resistant tuberculosis epidemics have increased the need for economical and reliable means of pathogen identification. There is also an urgent need to develop general hygiene monitoring methods that would allow rapid detection of microbial activity in health care and food industry facilities. The most important development in the field is the gradual emergence of DNA chip technologies that are expected to enable rapid and accurate detection of specific microbial strains.

Most of the diagnostic immunology assays of today are based on antibody-antigen reactions and relate to a wide variety of clinical conditions. For some immunology tests there are no reference materials establishing common standards, and results obtained from a single sample may vary depending on the experience and the competence of the operator. (Fleisher and Tomar 1997.) The targets of diagnostic immunology assays include lymphocytes (indicators of inflammation, leukemia and lymphoma), neutrophils (related to severe recurrent infections), and about 30 circulating protein complements that amplify and mediate inflammatory responses and indicate rheumatic diseases or severe inflammation. In the future the importance of tests related to the immune system is likely to increase further. The inflammatory response based on the interaction of cytokines (bioactive proteins released by B and T lymphocytes) and inflammatory cells is involved in medical conditions that are on the increase. The inflammatory response either works normally, is unsuccessful (in immunodeficiency syndromes), or is inappropriate (in autoimmunity diseases, allergy and asthma). (deShazo 1997.)

Diagnostic chemistry tests are used to measure the levels of hormones, pharmaceutical drug compounds, illicit drugs, and toxic compounds in the body. One of the challenges is to develop convenient methods for monitoring the levels of pharmaceutical compounds in human tissues so that treatments can be optimised on a continuous and individual basis.

Recent developments in haematology testing illustrate a technological trend that is likely to be manifested in many other areas of diagnostic technology in the future. The trend concerns the proliferation of portable and even hand-held devices producing rapid and reliable information. Clinicians value these devices because of their speed, reliability, and ease of use. Point-of-care diagnostic devices are available for glucose, complete blood count, cholesterol, calcium, blood urea nitrogen, potassium, chloride, haematocrits, sodium, haemoglobin, cardiac enzymes, and many other haematolological indicators. (Slomovitz *et al*. 1998.) One obvious future technological goal is to integrate several measurements in single, highly automated devices. Integrated point-of-care test panels would be greatly appreciated also in urinanalysis and in diagnosing heart conditions in emergency care units.

Tests related to human genetics are of recent origin, and for instance in the United States the diagnostics categories listed in the CLIA regulations of 1988 did not include a separate category for genetic tests. Until recently DNA tests were expensive and labour intensive. In the future DNA chips are expected to transform

the field. Already a chip capable of detecting all known mutations of the P53 gene, involved in cancer suppression, has been developed. (Provan 1999.)

4.3 Technological opportunities in diagnostics

New diagnostic methods often emerge from efforts to solve problems in biomedical research. At first the use of a new method requires specialised skills and is possible only in research settings. As a result of gradual technological improvements some variants of the method may be commercialised and can taken up in research hospitals and later still at general clinics. Factors constraining the speed of diffusion of new innovations include scarcity of skills, lack of appropriate regulations and standards, and bottlenecks existing in technological infrastructures that are required to support the new technology or link it with established procedures.

At the moment the supply of new medical diagnostic technologies is driven by the following six factors: new target analytes, the use of new bio-originated and other materials in sensors, miniaturisation, DNA technologies, automation, and information networks.

4.3.1 New target analytes

The number of medical diagnostic tests available in a typical hospital in Europe has increased steadily over the recent years and is estimated to be about 4 000 or 5 000. For reasons of economy a new test is likely to be adopted by a clinical laboratory only if it replaces an existing test or measures an entirely new function. However in a not-too-distant future the total number of available tests could reach 100 000, if each available DNA test will be counted as a separate test. In practice a great number of separate DNA tests will be carried out during a single procedure.

In a review of tests that have been recently added to the test menus of clinical laboratories it was discovered that many of the new tests were related to routine chemistry and profiling, thyroid testing, therapeutic drug monitoring, drugs of abuse, haemoglogin A1C, coagulation, and various microbia. Many of the new tests represented technological improvements over previously available methods; such improvement was given as a primary reason for 13 % of the new tests adopted by clinical laboratories of various types. New medical knowledge was cited as the

primary reason for instigating 11 % of the new tests. Advances in medical science and technology explained therefore directly only about one quarter of the adoption of new tests. Other primary reasons given to justify the adoption of new tests included the needs of optimal patient management as well as those of the community and the clients. (LaBeau 1998: 836-837.)

There is always a danger that a new test adds little of relevant information. One of the new emerging target analytes is a protein called homocysteine. Medical research since the late 1970's has suggested that an elevated level of homocysteine in blood is an indicator of cardiovascular disease risk. Tests costing between $40 and $150 have been developed for the measurement of the protein level, but critics have pointed out that the compound may actually be elevated after a heart attack or cardiac arrest, rather than before it. Three vitamins, folate, vitamin B12, and vitamin B6, degrade homocysteine. The reason why homocysteine is linked with cardiovascular disease could then be that low levels of these vitamins are a risk factor. (AACC 1998.)

One trend in medical diagnostic technology is an increase in the number of target analytes that can be measured in a single procedure. An ideal system capable of monitoring several target analytes at once could resemble the mammalian olfactory system, which can combine high sensitivity for individual substances with a broad range of odours detected and remembered. In the future metabolic illnesses such as diabetes may be diagnosed by analysing air exhaled by the patients. Future diagnostic technologies such as miniaturised gas analysers or electronic noses may be able to detect molecules associated with those diseases. Patients suffering from diabetes exhale small amounts of acetone that can be detected by an electronic nose based on a gas sensor array. (Ping *et al*. 1997) Similar techniques may be developed to monitor the health of husbandry animals.

Future artificial nose technologies could be applied in quality control in the food and processing industries as well as in environmental monitoring. One of their advantages is that they not only detect odours but can also be used to produce quantitative estimates of concentrations. That is why they have great potential in monitoring, for instance, carbon monoxide, other toxic elements, and amounts of combustible gasses (Göpel *et al*. 1998: 480). Problems to be solved include the high cost and large size of present devices, need to regulate humidity and temperature, sensor poisoning, as well as poor repeatability and sensitivity. (Dickinson *et al*. 1998: 256-257). In a distant future it may become possible to incorporate in

artificial devices mammalian olfactory receptor proteins produced in bacteria (Göpel *et al*. 1998: 486).

While electronic noses of the future will be able to monitor gaseous environments, similar capabilities are going to be build into devices that monitor liquids. Such electronic tongues could be useful in medical diagnostics, food industry, processing industry and in monitoring industrial effluents. Devices intended for food industry applications include systems that have miniaturised sensors for sweet, sour, bitter and salty compounds. Future applications in point-of-care diagnostics could produce comprehensive information from blood or urine samples. (Toko 1998.)

4.3.2 New sensor-surface materials

In diagnostic applications sensors offer several advantages compared to conventional reagent-based analyses. Sensors save the costs of purchase and storage of reagents, require small samples, and produce results rapidly. Sensor-based diagnostic systems are particularly desirable in point-of-care applications.

In biosensors the detection of target substances is based on biochemical interaction between a target and a biological compound immobilised on the sensor surface. The detecting biological material may consist of an enzyme, antibody, receptor, nucleic acid, a whole cell, or in the future even of a part of a sense organ of an animal or insect. The main advantages of biosensors are their specificity, speed and low cost. Durability, reliability, quality, and calibration are more problematic and call for new methods of manufacturing biosensors. Immobilisation of biological components used in biosensors is usually not easy. Most biosensors cannot withstand sterilisation procedures. Sometimes it is difficult to design the interface between the biological detector and the transducer.

Antibodies and enzymes are the most common biosensor surface membranes. Glucose oxidase and urease enzymes in conjunction with pH or oxygen electrodes were among the first biosensor applications. The combination of oxidoreductases and amperometric electrodes is regarded as the most promising combination also in the development of future biosensors. There already are sensors of this type available for about 80 analytes. (Luong *et al*. 1997: 372.) Glucose monitoring is the most common diagnostic application of biosensors, and about 90 % of world’s

biosensors are glucose sensors; yet electrochemical devices remain the dominant technology. Permanent biosensor implants for glucose measurements have also been developed. (Wilkins and Atanasov 1996.)

The two main methods of receptor molecule design are the rational approach and the use of combinatorial chemistry, where cyclopeptides have been recognised a particularly rich source of potential receptor molecules. (Ziegler *et al*. 1998: 550-552.) Promising receptor molecules can also be modified to improve their performance. Bio-originated antibodies and enzymes can be modified by modifying the genes that encode them (Rapley 1995).

Sensors based on natural receptors are often called bioaffinity sensors. Their targets are molecules that fit into the receptors like a key in a lock. Bioaffinity sensors can be mimicked by preparing synthetic epitopic receptors in which the crucial molecular binding sites are identical with the natural receptor. For instance, it is possible to prepare polymer structures that mimic antibodies and are able to bind their corresponding antigens. In this method a polymer surface structure is made to carry functional groups of monomers to precise locations in which they are able to form a complex with the target molecule. The method is known as molecular imprinting, and it will probably make it possible to develop sensors for a great variety of purposes. (Haupt and Mosbach 1998: 469.) Future applications of the technique may include recognition of undesirable compounds, including environmental pollutants and herbicide residuals (Whitcombe *et al*. 1997).

In the long run it may become possible to develop general-purpose environmental safety or hygiene-monitoring devices based on biosensors. Such biosensors could act as whistleblowers indicating that there is a need to carry out more accurate analyses that would pinpoint the exact cause of the alarm. For hygiene monitoring general purpose methods have already been developed. One of the methods relies on the detection of the energy carrying ATP (adenosine triphosphate) molecules with the help of Luciferase firefly enzyme. The enzyme catalyses a reaction in which one photon is released for each ATP molecule reacting with the enzyme. The resulting low-level bioluminescence can be detected and measured as an indicator of microbial presence. (Hawronskyj and Holah 1997.) Microbes can be used as biosensors to detect known and unknown toxins, but calibration and reversibility can be expected to be a problem (Ramanathan *et al*. 1997: 501).

Cells, their organelles, and parts of tissues may in the future be engineered to be used in biosensors and more comprehensive diagnostic systems. Their use can be expected to be limited mainly to research purposes. Cells from sense organs and neurons performing sensory data processing tasks are particularly interesting sensor materials. Cells of the olfactory epithelium have been co-cultured with cells from the olfactory bulb of the brain. Functional connections from the epithelium to the brain cells have been observed to develop, and further tests will be carried out with odourants to see whether they activate the functional groups. (Ziegler *et al*. 1998: 559.) Cultivated high-level neurons from the crayfish olfactory system seem to respond to odour stimuli (Mellon and Alones 1997).

Biosensors based on nerve cells are at an early research phase. So far non-sensory nerve cells can be cultivated to form a fault tolerant system that becomes spontaneously active and exhibits considerable sensitivity to the chemical environment. Networks of cultivated neurons have been maintained in a physiologically active and pharmacologically responsive state for over nine months. Monitoring of metabolism, synaptic activities (including neurotransmitters) and other physiological indicators of such living networks makes it possible to use them as sensors. Certain substances have been observed to elicit specific electrical responses from the neuron networks. For instance, the gp-120 protein of the AIDS virus seems to produce unique electrical discharges. (Ziegler *et al*. 1998: 557-558.) In the future it may become possible to use living neuron networks in detecting not only known compounds but also substances whose presence cannot be anticipated (Hickman 1997). Traditional techniques can be employed for the exact identification of substances causing unusual reactions in neural cell sensors.

For research purposes methods would be required for the monitoring of intracellular processes. Diagnostic methods based on intracellular probes using, e.g., proteins marked with fluorescent molecules have already been developed. Some of the probes relate to the programmed cell death or apoptosis. (Giuliano and Taylor 1998: 137). In research environments the production of protein probes can be programmed into the host DNA. The technique has already been used to grow genetically encoded fluorescent protein probes that can be used to measure transmembrane voltages within single neuron cells. Voltage and other sensors encoded into DNA have the advantage that they are introduced noninvasively into an organism and can be targeted to specific developmental stages or sites, such as brain regions, cell types, and subcellular compartments. (Siegel and Isacoff 1997.)

4.3.3 Miniaturisation

Miniaturisation is a key technology enabling the development of clinically relevant and versatile point-of-care diagnostic systems. Miniaturisation also makes it possible to design increasingly integrated diagnostic laboratory systems. Miniaturisation extends to diagnostic imaging: prototype hand-held ultrasound scanners have already been developed. Miniaturised systems would also have potential in industrial and environmental monitoring. On the other hand future analysis methods designed for industrial machine vision and spectroscopy could find applications in medical image analysis as well.

Miniaturisation of diagnostic analysis systems aims at speeding analyses, reducing reagent consumption, allowing smaller sample sizes and enabling the development of modular systems that can be combined for the rapid automated performance of complex analyses. Future analytic systems that have been reduced to chip size can be expected to be inexpensive to manufacture in large quantities. In some cases the costs may be reduced to such extent that chips can be disposed of after use.

There already are portable devices that produce results as sensitive and selective as those obtained in full-size laboratory systems. In blood analysis the use of point-of-care devices helps to avoid errors resulting from ongoing metabolism and electrolyte movements during transport of the sample. The convenience of point-of-care analysers is added by the fact that they do not necessarily require, for instance, the separation of blood cells and plasma. A recently introduced portable blood gas and electrolyte analyser was evaluated to perform comparably with established laboratory systems. (Lindemans *et al*. 1999: 111.)

Another indicator that can now be measured by using a hand-held device is the level of blood lactate. The measurement indicates the severity of injuries sustained by critically ill trauma patients. The measurement takes about 60 seconds, a considerable improvement in trauma situations, as the conventional laboratory procedure takes about one hour. (Slomovitz *et al*. 1998.) Yet it has to be recognised that advances like this do not always manifest themselves in numeric indicators of clinical performance. In a recent study at an emergency department of a large hospital it was discovered that point-of-care blood testing reduced the time it took to adjust treatment in cases in which timing was considered to be critical. However there was no statistical improvement in the clinical outcome nor any reduction in the time patients spent in the emergency department. (Kendall *et al*. 1998.)

Advances in micro-electro-mechanical systems (MEMS) have created a solid basis for future miniaturisation of diagnostic systems. In the future combinations of miniaturisation techniques taken from the semiconductor industry with techniques emerging in biochemistry, antibody technology, and DNA analysis are likely to result in various 'laboratory on a chip' concepts. Fluid channels, heaters, temperature sensors, fluorescence detectors, and preset reagents can already be built on silicon chips. Such constructs will enable the development of automated miniaturised laboratory procedures. (Burns *et al*. 1998: 484.) The laboratory-on-a-chip technology will give an added boost to the diagnostic use of antibodies, as a single properly prepared sample can be subjected to wide-ranging antibody microassay on a single chip. The first miniaturised diagnostic testing kits available are based only on a few antigen-antibody reactions and target a few bacteria, viruses and toxins. (Borriello 1999.)

It is likely that future integrated microfluidic analytical systems based on silicon or quartz chips will be increasingly used in point-of-care analyses. Wafers that are being developed at the moment distribute a liquid sample into 8 or 16 channels. In each channel a separate assay can be performed with preloaded reagents. New opportunities for the future may arise from the use of mass spectrometry and electrophoresis technologies, which have already been implemented on microchips. The numbers of channels can be expected to increase in the future, and it is also likely that optics, light sources, sensors and other components of diagnostic systems will be miniaturised or even integrated in the chip constructs. Such devices will allow the realisation of latent demand for rapid, inexpensive point-of-care analyses. Moreover, it is increasingly recognised that some of the future systems may be suitable for home use as well. (Regnier *et al*. 1999: 101.)

4.3.4 Diagnostic technologies based on DNA chips

DNA chips are fingernail-sized silicon or glass surfaces on which known sequences of DNA nucleotides from pathogens or gene mutations have been immobilised by using lithographic or ink-jet-printing techniques. DNA strings on the chip are marked with a fluorescent label and combine with corresponding strains from the sample. The combinations can be detected by using optical methods.

One indication of the importance of the DNA microarray technology is that according to some estimates future revenues from the sales of biochips could rival those from computer chips (Schena *et al*. 1998: 302). The likely impact of DNA and antibody chips on medical diagnostics can be likened to what semiconductors did in electronics: some of the sophisticated laboratory bench systems of today may face the fate of mainframe computers of the past. The combination of modern semiconductor technology and molecular biotechnology in DNA chips is likely to lead to a new dominant design in diagnostic technology, revolutionise diagnostic product markets, and transform the competitive conditions in the industry.

In medical diagnostics, DNA chips will allow extraction of a large amount of information quickly from a single saliva, urea, stool, blood, or tissue sample. (Hoheisel 1997: 468) Future DNA chips are likely to contain tens of thousands of DNA strings that can identify genetic polymorphisms, mutations and pathogen strains. The chips can be expected to be simple and rapid to use as well as inexpensive due to mass production and minute labelling chemical requirements. In the future it may become possible to interpret the results of DNA chip tests by using a portable device. It is expected that the integration and automation of all sample handling and measurement steps into a single package will make the miniaturised systems ideal for certain types of medical tests in health care. (Blankenstein and Larsen 1998: 427) The promise of miniaturised biomedical technologies is illustrated by the fact that a miniaturised DNA sequencing apparatus has already been designed (Foote 1997: vi).

As diagnostic tools DNA chips can be expected to have numerous applications in medical conditions that involve a genetic component. The chips will undoubtedly help also in the diagnosis of cancers and diseases caused by pathogens. In the long run the chips may also be used in tailoring the mix of pharmaceuticals to fit with the particular genetic makeup of an individual, a particular pathogen or a type of cancer. Certain polymorphisms are already known to affect the efficacy of some drugs. Future genetic screenings could indicate on which patients certain pharmaceuticals are likely to have adverse side effects. (Graves 1999: 128.)

4.3.5 Automation

The main focus of automation in clinical laboratories has been in increasing the efficiency of analytic processes. Preanalytic and postanalytic phases have proven more difficult to automate, but they are expected to receive more attention in the future. It is at these phases where bottlenecks are now found in the specimen flow process and highly repetitive manual tasks still take place.

The main benefits of laboratory automation relate to the personnel costs, work volume, and quality. One report concluded that during a 18 month period computerisation of a laboratory led to a 15 % reduction in staff while the test volume doubled. After the preanalytical phase was automated by introducing a modular robotic system, a further 17 % saving in personnel costs was achieved. (Dadoun 1998.)

Modular automation allows stepwise elimination of the most tedious tasks in a laboratory, while fully automated systems perform all or most of the tasks related to analyses. Control system software is of special importance as the status of individual samples has to be carefully monitored, each analytic step recorded, results stored and retrieved, and problems rapidly detected and reported to the personnel.

In the future advances in computerised image analysis could affect many areas of diagnostic testing. One of the tests in which microscopic study of the sample is necessary is the Papanicolaou (Pap) test in screening for uterine cervical cancer. Routine Pap screening is tedious, and abnormal cells an be missed because of the monotony of the work. Automated rescreening devices can be used to select slides for human review. There are prescreening devices as well, and primary screening systems, capable of replacing human inspection, are being developed. Yet it has been cautioned that progress can be expected to be slow. (Rosenthal 1998.)

Neural networks have been one of the main software methods studied for the purpose of developing automated image analysis systems. Diagnostic applications in which self-organising maps may prove to be helpful include interpretation of electrocardiograms (Abreu-Lima and de Sa 1998), detection of false negatives in microscopic screenings for cancer cells (Mango 1996), and classification of magnetic resonance images (Reddick *et al.* 1998).

Diagnostic algorithms represent a new phase in medical laboratory automation. Algorithms can be likened to automatic reflexes arising on the basis of findings obtained in primary tests. Algorithms launch corroborative or secondary tests

automatically. Their aim is to reduce the time it takes for a doctor to arrive at an accurate diagnosis by providing a more comprehensive set of information than what was obtained on the basis of the primary tests alone.

4.3.6 Information networks supporting diagnostic systems

Health care has lagged behind many other sectors of society in adopting automation and the use of information networks. One of the main reasons is that even the present high capabilities of PC computers and multimedia networks are not sufficient for typical medical applications. A radiologist needs to view simultaneously several high-quality images when preparing a diagnosis. The cost of the required high quality displays, workstations and data networks would be very high. That is why the development and adoption of digital radiological picture archiving and communication systems (PACS) has been a relatively slow process. Similarly voice recognition capabilities of the present computer systems cannot replace typists in converting voice dictations into patient records that can be properly archived.

The present software and hardware technologies tend to constrain information that can be stored in computerised patient records. Attempts have been made to standardise clinical vocabulary so that patient records could be encoded for later information retrieval. These efforts have not been successful. (Wang and Wang 1999.) Clinicians tend to use many different phrases to describe the same clinical concept. That is why free-text based systems may be an appropriate choice for the future. (Powsner *et al.* 1999.) Information systems have to become more flexible and intelligent in order to properly serve the needs of health care professionals; it would be absurd to try to constrain the routines of health care professionals so that they would serve better the needs of an information system.

Fortunately hardware and software technologies continue to develop rapidly. That is why we can expect that in the long run electronic patient records will contain data-intensive multimedia files including radiological images. Even before then electronic access to patient records will improve the quality and economic efficiency of patient care. Future diagnostic systems should be able to store diagnostic information directly in databases for later reference. The huge amounts of genetic information that can be obtained in the future increases the urgency of developing information systems of high capacity.

There is an increasing need to integrate various health care databases and to take advantage of opportunities created by local, national and international data networks. One of the main aims of aiming at database integration is to improve decision support for clinicians. (Rivers and Bae 1999.) It would be important to develop systems that would be able to combine diagnostic information with decision support tools that relate the findings to information gathered on wider populations and in recent research. Even independent medical doctors could be provided with access to electronic patient records by using a smart card that the patient carries. It is likely that the most convenient and cost-efficient way of realising such services is to use the increasingly ubiquitous IP and other Internet-related standards in combination with secure virtual private network technologies.

In the future information networks will be utilised in ordering tests from a laboratory, retrieving results, and in managing and updating patient records. (Athena Society 1996: 98.) Information networks will support and extend laboratory automation by allowing direct transmission of test results from automated medical diagnostic systems to computers at the doctor's office or to hand-held communicator devices at the hospital bedside. Efficient information networks would enable contacts between the doctor and an automated diagnostic system. The doctor could signal the need for confirmatory and secondary tests before they are carried out. Thus information networks could lessen the need for endless development of algorithmic testing procedures while also enabling rapid performance of secondary tests on the original sample.

4.4 The demand for new diagnostic technologies

The demand for new diagnostic technologies is driven by novel discoveries in biomedical research. As soon as *Helicobacter pylori* was accepted as the main cause of gastric ulcers a need arose to diagnose the presence of the bacteria. In one study 38 % of clinical laboratories in the Pacific Northwest of the United States added *Helicobacter* antibody tests to their test menus between 1994 and 1996 (LaBeau *et al*. 1998: 837).

The demand for new diagnostic test technologies is constrained by economic factors. In order to be adopted, a new diagnostic test has to be recognised as offering either new important diagnostic benefits, functional advantages, such as automation, or allow the phasing out of more costly or less reliable alternatives.

At the moment the demand for new technologies in diagnostics is fuelled by several factors: trends in diagnostic laboratory management, the special needs of the main groups of users of diagnostic medical technology, the need for further automation, and the necessity to integrate information gathered during various phases of the diagnostic process.

4.4.1 Trends in diagnostic laboratory management

Future demand for diagnostic systems and devices is influenced by such factors as consolidation of laboratories, networking, outsourcing, automation (including robotisation), downsizing, and increasing importance of point-of-care testing. The impact of consolidation can be illustrated by figures from the United States. In 1970 there were more than 9 000 independent clinical laboratories in the country, but by 1997 the number had declined to less than 4 000 laboratories (Jahnle and Rebane 1997).

Consolidation of laboratory services enables investments in automation and reduction of costs per laboratory analysis. Networking collaboration creates similar benefits, as laboratories are able to specialise. Additional benefits of networking include savings in co-operative equipment purchases, maintenance, and personnel training. While centralisation of some types of tests offers economic benefits, the immediate diagnostic value of some tests is such that they need to be carried out at point of care. At present tests that are deemed essential for optimal patient care and need to be performed at physicians' office laboratories include urinanalysis, urine sediment, urine pregnancy tests, microhaematocrits, complete blood and leukocyte counts, as well as tests of glucose and haemoglobin concentrations (LaBeau *et al*. 1998: 837).

4.4.2 Requirements of users of medical diagnostic technologies

The are four main groups of users of medical diagnostic technologies. It can be expected that in the future independent laboratories will take advantage of economies of scale and will concentrate on analyses that can be automated and are not urgently required in patient care. Hospital laboratories will take care of analyses the results of which are urgently required for diagnosis and therapeutic decision making. Rapid and easily administered analyses will be carried out at the bedside

within hospitals and in the practices of independent medical doctors. The last group of users consists of patients themselves and their families. Home users need to be able to carry out tests that are useful for health monitoring and in supporting the treatment chronic illnesses over long periods of time.

The technological requirements of the four types of users of diagnostic technologies differ markedly from each other. Independent central laboratories will base their competitive strategies on cost and logistic efficiency. They will be able to reduce costs by investing in increasingly high levels of automation in handling samples, reporting results, and even in carrying out secondary tests in order to increase the relevance of their work for clinical decision making. As a result of the high level of automation, central laboratories can be expected to achieve very high quality levels in terms of reliability of results.

Hospital laboratories will continue to enjoy the logistic and perhaps also the cost accounting benefits of operating within patient care organisations. They are flexible in serving hospital needs and in giving diagnostic advice to the staff. Depending on the volume of tests and co-operation arrangements hospital laboratories will be able to invest in automation and offer valuable specialist services. In public health care hospitals are the natural repositories for future databanks storing patient records and the results of previous examinations and analyses. The ability to combine electronically historical patient information with the results of most recent analyses could give important benefits to health care and hospital economy.

Point-of-care diagnostic systems are required at the bedsides of hospitals and at the offices of independent medical doctors. In cost comparisons point-of-care testing has fared surprisingly well (Hoerger and Meadow 1997). High reliability, simplicity of operation, rapid availability of results, low cost, and portability are important factors in the selection of point-of-care diagnostic systems. Point-of-care diagnostics benefits from a technological trend that turns expensive specialist instruments of today into tomorrow’s inexpensive and simple-to-use point-of-care or home devices.

Another requirement in point-of-care testing is a need for minimally invasive methods. Tissue samples should be replaced with blood samples, when possible; urine, saliva, hair and fingernail samples should be used instead of blood samples. In the future new methods may be developed, which require no samples at all. They could include laser-based skin analysers as well as gas analysers or artificial noses performing automated analyses on exhaled air. Methods based on monitoring

electronic, acoustic and magnetic phenomena of the body may be also refined to yield increasingly revealing information about various ailments. A non-invasive glucose monitoring device patented in Japan in 1997 consists of a suction cup containing a semiconductor laser for light emission and a light detector for determining the level of glucose in blood circulation under the skin (Fujii *et al*. 1997: vi). Optoelectronic devices may become a favoured solution in non-invasive in vivo diagnostics.

In home use the present popularity of weight, blood pressure, and heart rate measurement devices indicates that there could be considerable demand for more sophisticated health monitoring instruments. Another indication that points to the same direction comes from the popularity of fitness monitoring devices. VTT Information Technology and its industrial partners recently developed a prototype of a personal health monitoring system. The portable system is based on a laptop computer and includes instruments for measuring body temperature, blood pressure, body weight, breathing rate, and heart rate. A portable recorder collects data on heartbeat rates through the day, and during the night monitoring is carried by a sensor placed under the mattress.

4.4.3 The need for further automation

The need for further automation is evident in diagnostic examinations that still are labour intensive. One of the fields in which advances in automation are urgently required is computerised image analysis. Automated methods of the future could improve the quality and productivity of many areas of diagnostic testing that rely on manual clinical microscopy and human interpretation. Such tests are carried out in urinalysis, haematology, and cytology.

The microscopic examination of cells found in urine is time-consuming and costly procedure. Moreover, the counting of various cells is time-consuming, tedious and prone to error. Because of these problems, the examination is often discarded, if the results of chemical screening with dipsticks are negative, although this rule of thumb may lead to erroneus conclusions. Because of these reasons attempts are being made to replace microscopic examination with automated methods, such as flow cytometry. (Langlois *et al*. 1999: 118.)

In flow cytometry thousands of cells per second flow through the device in a suspension liquid and are illuminated by a laser; the light scattered from the cells is detected by photomultiplier tubes, enabling the measurement of some cell properties. In the future the method may be increasingly used in semen analysis as well. At present semen analysis, too, is carried out by laboratory operators using microscopial inspection. (Ferrara *et al*. 1997: 801.) Flow cytometry may also be applied in the future in preconceptional sex selection for human and animal offspring. In additional to ethical issues the risks include damages caused by DNA stains and ultraviolet light. (Hossain *et al*. 1998.)

4.4.4 The need for information integration

Integration of information obtained in diagnostic analyses with information from electronic databanks, various information services, and patient records will add a whole new dimension to the diagnostic process of the future. Future diagnostic systems should automatically save the results of analyses for future review by using automated wired or wireless links to information databases. Historical data can help to reveal important information about trends in indicators that are being monitored at the request of a doctor or by the volition of the individual.

In hospitals diagnostic laboratories can use information systems to assist physicians in the interpretation of test results. In one experimental data repository system haematology, coagulation, diagnostic immunology, and clinical chemistry test results are brought together with pathology reports and clinical notes. It has been discovered that the system helps to eliminate redundant diagnostic testing, screen for hospital-acquired infections, and even identify changes in the prevalence of antibiotic-resistant bacteria strains. (Saltz *et al*. 1998.)

The combination of database and information networking technologies with consumer diagnostic devices could result in sophisticated services for those sections of the population who are either very interested in health matters or have medical conditions that need regular monitoring. Application of data networks in conjunction with diagnostic devices allows not only guidance to the user, information storage, and trend analysis, but also the use of automated alarms when developments are detected that require professional attention. The first commercial systems are being developed and are aimed at serving the needs of asthma and diabetes patients.

The need for integrating information can at times be solved by integrating several analyses into a single diagnostic system. Miniaturised arrays of tests not only save costs and time but also allow rapid integration of information. That is why panels of tests are useful in many real-life situations such as in allergy testing, screening of transfusion blood, forensic science, and environmental analyses. As diagnostic arrays are becoming increasingly complex and may contain in the future tests based on chemical reagents, antibodies, and DNA, the user interface becomes an increasingly important part of the system. Software used in the system should be able to highlight the most salient findings and suggest interpretations.

Computerised information processing can be used to combine information arising from various sources and various types of sensors. In applications in which both gaseous and liquid targets are to be monitored, an artificial nose can be integrated with sensors suitable for analyses of liquids, and the resulting information can be put together in an information processing system. (Dickinson *et al*. 1998: 250.) Diagnostic systems of the future may include algorithms that will be able not only to store information but also recognise emerging patterns and learn.

4.5 Conclusions

Medical diagnostic technology comprises techniques and methods used in the detection and quantification of physiological and medical indicators of human health. Diagnostic systems take advantage of several technologies combined into a seamless whole. Many of the constituent technologies of medical diagnostic systems are advancing at a rapid pace; as a result, a great number of technological opportunities are emerging in medical diagnostics. The success of companies and research teams in the field depends on their ability to secure access to expertise on biotechnology, electronics, automation, and information systems.

Already in the short run individual diagnostic devices with startling properties will emerge in medical diagnostics. Miniaturised test kits will become available for some of the most common haematology and bacteriology tests, which can then be performed at the bedside without referring samples to the clinical laboratory. DNA chips will become increasingly available for various research applications.

In the medium run it will become possible to develop improved interfaces between diagnostic devices and information networks in hospitals. It will become

increasingly common to feed information gathered by diagnostic devices directly into databases for later referral. Miniaturised diagnostic kits will rely on wireless connections, while large automated systems in the laboratory will take advantage of fixed networks. Efforts will be made to integrate information systems storing radiological images, MRI scans, EKGs, reports from clinical laboratory analyses, and administrative records. Laboratory automation advances at a steady pace and is going to become more comprehensive; automated systems will be able to communicate with the doctor before carrying out confirmatory or secondary tests. In the medium run tests based on DNA chips as well as chips based on large numbers of antigen-antibody reactions will become available for general use in hospitals.

In the long run a new diagnostic infrastructure is likely to take shape. It will be based on national and international information networks connecting databases of research findings and patient records, decision support tools, automated laboratory systems, individual diagnostic devices, doctors' work stations and even the homes of patients who themselves will be able to collect some of the information and also access parts of it. The build-up of the diagnostic infrastructure will be driven by the need to store and access huge amounts of radiological, DNA and other diagnostic information. Information networks facilitate transfer of information collected for instance at the site of an accident, at various health care and hospital locations, and even at the home of the patient. Moreover, doctors require improved collaboration and decision support tools in making clinical decisions on the basis of increasingly complex and detailed diagnostic information. Integrated diagnostic networks will also improve the ability of patients to manage chronic conditions at home.

5 Pharmaceuticals

5.1 Biopharmaceuticals vs. pharmaceuticals

In surveying opportunities in pharmaceuticals our focus will be on mapping out opportunities in drug development, presenting examples of promising research directions, and describing the basic features of competitive conditions in the pharmaceuticals industry. The impact of biotechnology on pharmaceuticals is mainly based on biotechnical methods that can be applied in research, development and manufacturing.

In the future biotechnology will be used increasingly in pharmaceutical production, and biopharmaceuticals, defined as drugs manufactured by using biotechnological means, will be given close attention in this report. The market share of biopharmaceuticals started from a low level in the early 1990's, but is rapidly increasing in the international medical drug market. Biopharmaceuticals can be expected to constitute about 10 % of the pharmaceutical market at the end of the decade. Most biopharmaceuticals are based on large and heavy molecules, often proteins or glycoproteins, while many traditional synthetic drugs are products of low molecular weight. The methods used in the identification, development, production, and delivery of biopharmaceuticals often differ dramatically from those in traditional pharmacology.

Drug delivery is a typical problem of biopharmaceuticals. It is not easy for large molecules to penetrate various chemical barriers in the body. That is why efforts to develop biopharmaceuticals need to be supported at an early stage with studies on means of efficient drug delivery. Because of problems of drug delivery, many biopharmaceuticals may eventually be replaced by synthetic small-molecule compounds. In many cases, though, the replacements will take advantage of the particular shape of the pharmacologically active region of the original biopharmaceutical molecule.

5.1.1 Biopharmaceuticals of today

The first scientific drug discovery efforts concentrated on evaluating natural compounds that might be used in pharmaceuticals; the emphasis was on extracting beneficial bio-originated ingredients from plants and other sources. During the first

half of the 20th century, systematic pharmacological evaluations of both synthetic and natural compounds were carried out. After the usefulness of a candidate compound was ascertained, a synthetic process was developed for its production.

Advances in antibody biology and gene technology placed the drug discovery process on a more rational footing in the 1980's. It became possible to design drugs for specific molecular receptor sites and protein abnormalities, including deficiencies in enzymes. The development of computerised methods has further increased the opportunities for rational drug design (Kaul 1998). The power of the rational approach is confirmed by the increasingly accurate biomolecular explanations discovered for the efficacy of existing, experimentally developed drugs. For instance, it has been discovered that the reason why aspirin helps to reduce inflammation is that it protects a protein that inhibits the onset of the inflammatory process (Yin *et al*. 1998). Such explanations help to develop further and ascertain rational drug design principles.

It has been estimated that there are already more than 65 biotechnical drug products on the market. About 300 more biotechnical drug and vaccine products are in human clinical trials. (Feldbaum 1998.) Biopharmaceuticals can be used to treat diseases such as anaemia, cystic fibrosis, dwarfism, hairy cell leukemia, hemophilia, and Kaposi's sarcoma. Some of the available products are listed in Table 5.1. In gene therapy recombinant DNA technology is applied to correct single-gene hereditary disorders. New types of vaccines are also being designed by applying transfected DNA expression of antigen molecules.

Table 5.1. Some biotechnological medical products and their dates of approval in the United States.

Biopharmeceuticals approved for use in the United States			
Product	**Company**	**Application**	**FDA approval**
Humulin® (recombinant human insulin)	Eli Lilly	diabetes	Oct. 1982
Novolin® (recombinant human insulin)	Novo Nordisk	diabetes	Oct. 1982
Protropin® (somatrem)	Genentech	growth hormone deficiency in children	Oct. 1985
Albutein® (human albumin)	Alpha Therapeutic Corp.	treatment of hypovolmeic shock; an adjunct in hemodialysis	Jan. 1986
Recombinate® (recombinant antihemophilic factor)	Baxter Healthcare	blood-clotting Factor VIII for the treatment of hemophilia A	July 1986
Intron A® (alpha-interferon)	Schering-Plough	hairy cell leukemia	June 1986
Roferon-A® (recombinant interferon alfa-2a)	Hoffmann-La Roche Inc.	hairy cell leukemia	June 1986
Recombivax-HB®	Merck	recombinant hepatitis B vaccine	Jan. 1987
Tripedia®	Pasteur Merieux Connaught	immunisation of infants primarily for whooping cough	Nov. 1992
Betaseron® (recombinant interferon beta 1-B)	Berlex Labora-tories/ Chiron	relapsing, remitting multiple sclerosis	Aug. 1993
Nutropin®/ Nutropin AQ® (somatropin rDNA)	Genentech	growth hormone deficiency	Nov. 1993
Pulmozyme® (dornase, alfa recombinant)	Genentech	cystic fibrosis	Dec. 1993
DaunoXome® (liposomal form daunorubicin)	NeXstar Pharmaceuticals	treatment for HIV-related Kaposi's sarcoma (liposomal drug delivery)	Apr. 1996
Humalog® (recomb. insulin)	Eli Lilly	diabetes	June 1996
TriHIBit™	Pasteur Merieux Connaught	childhood immunisation for acellular pertussis, diphtheria, tetanus and HIB	Sept. 1996
Zenapax (Daclizumab)	Hoffmann-La Roche Inc.	humanized monoclonal antibody for prevention of kidney transplant rejection	Dec. 1997

Source: Biotechnology Industry Organisation. (http://www.bio.org/whatis/guide2.html).

5.1.2 Competitive conditions in the pharmaceutical industry

Compared to many technologically mature industries the world pharmaceutical market is not consolidated to a significant degree. No single pharmaceutical company has a share of over 5 % of the $300 billion market. This would indicate that relatively free international competitive conditions prevail within the industry, but in individual markets related to particular medical conditions the market position of leading companies is often strong.

The drug compounds of today interact with only about 400 molecular targets in the human body. The total number of effective pharmaceutical compounds listed in Finland is about 900 (Lääketietokeskus 1999). These surprisingly low figures by themselves suggest that among the millions of chemical interactions within the human body there must be unexploited targets for new medical compounds.

In 1998 only 35 new molecular entities (NMEs) were launched on the world market. Yet during that year alone altogether 11 000 pharmaceutical R&D projects were being carried out by the pharmaceutical industry. Over 3 600 projects had reached clinical phase. Neurological therapeutic substances had a 13 % share of the R&D projects, cancer drugs 11 % and the share of potential anti-infective drugs was 9 %. (Financial Times 1999.)

The cost of developing new pharmaceuticals is high. In the United States, the Office of Technology Assessment estimated in 1993 that for drugs that first entered human testing in the period 1970-1982 the fully capitalised cost of developing a new pharmaceutical was $359 million in 1990 dollars. For drugs introduced in 1990, the cost was $500 million (PhRMA 1998); one recent estimate is $400 million (Gilmartin 1998).

It has been estimated that only one compound in 10 000 will succeed as a new pharmaceutical, and that the process takes approximately 12 years (Michels *et al*. 1998: 211). Efforts have been made to streamline the drug approval process while maintaining the high level of safety that has been achieved on the basis of existing regulations. In the United States The FDA Modernization Act of 1997 was the first major legislative reform of food and medical product regulation in 35 years. A wider access to experimental drugs and medical devices was allowed by the reform,

and filing and approval procedures of new therapies for serious or life-threatening conditions were streamlined. (FDA Consumer 1998.)

Ultimately the high cost of medical research reflects a need to improve the methodologies of drug discovery and development. One reason for the high costs is the risk of discontinuities between *in vitro* and *in vivo* results. Similarly, preclinical animal models do not always eliminate the chance of negative results in clinical studies. The development of more accurate molecular, cell, and animal models is therefore a strategic priority for pharmaceutical R&D. Rapid advances in the Human Genome Project, drug screening methods, DNA chips, and computer modelling techniques are likely to increase the rate at which new original drug compounds are discovered. The costs of pharmaceutical research could also be reduced if it could be determined at an early stage of research whether it is possible to develop suitable drug delivery methods for lead compounds that are interesting in other respects.

The process of drug development is becoming increasingly systematic and computerised resulting in gradual cost savings. Subsets of human genes, such as thousands of those involved in cell-to-cell communication, can already be identified, and their proteins tested in high-throughput systems to clarify their functions and discover potential medical applications. Such an approach was used recently to show that a compound, BlyS, has potential for stimulating the human immune system against a variety of infectious diseases and strengthening the protective impact of some vaccines. (Moore *et al*. 1999.)

Large pharmaceutical companies, including those listed in Table 5.2, are the financial engines driving the R&D efforts of the industry. In 1998 some of the largest pharmaceutical companies were carrying out between 100 and 200 research projects each. Yet more than one third of new medical compounds nowadays originate from relatively small companies that have specialised in pharmaceutical R&D. The number of such companies is about 600 in the United States and 300 in Europe.

Table 5.2. Market capitalisation of the largest pharmaceutical companies in the world in 1998.

Company	Market capitalisation ($ bn)
Merck	198.0
Pfizer	179.9
Bristol-Myers Squibb	126.7
Novartis	122.9
Roche	116.6
Eli Lilly	105.0
Schering-Plough	82.9
American Home Products	82.0
Smithkline Beecham	80.7
Zeneca[1]	38.6
Astra[1]	32.3
Monsanto	27.8
Pharmacia & Upjohn	27.5
Bayer	24.7
Hoechst[3]	24.6
Sanofi[2]	20.8
BASF	19.9
Rhône-Poulenc[3]	16.8
Synthélabo[2]	10.8

Market valuation on March 10, 1999. Planned mergers indicated by superscript (1, 2, 3). Source: Financial Times, March 31, 1999.

Ideas for possible NMEs often first emerge in academic research carried out at universities. The ideas are taken up either by R&D companies or by members of the research teams who try to commercialise the new findings themselves. The overall result is an intricate network of co-operative arrangements sustaining wide-ranging research efforts even though only a small proportion of the projects lead to new commercially successful pharmaceuticals. Large pharmaceutical companies, as well as public and private financing institutions, have a rational incentive to spread the risks of R&D and support research on a large number of leads.

5.2 Pharmaceuticals of the future

5.2.1 Targeting the genetic component of human disorders

The human genome consists of about 80 000 genes stored in a total volume of three billion chemical base pairs of DNA. The Human Genome Project aims at completing an accurate sequence of the human genome by the end of the year 2003 (Collins *et al*. 1998). At present the number of inherited diseases is estimated to be about 4 000. In the future the figure may be considered to be higher as hereditary cofactors may be recognised to be involved in diseases and conditions in which pathogen contagion, environmental factors, or even behavioural habits, play a dominant role.

So far gene therapy methods have been developed for the treatment of a few single-gene disorders such as cystic fibrosis and severe combined immune deficiency. In the future success may be achieved in developing treatments for polygenic diseases in which there have been mutations in several genes. It will be even more difficult to find treatments for multifactorial genetic disorders resulting from genetic factors and long-term environmental influences. Due to financing and human constraints science is at its weakest in solving puzzles that relate to slow changes that take place over decades. The complexity of research tasks is increased by the existence of genetic polymorphisms, or slight genetic variations that influence the properties of expressed proteins in special circumstances, and by the fact that some genes have more than one function. Fortunately technologies used in the identification of genes and in the clarification of their functions are developing rapidly.

Table 5.3 presents the most common methods of gene discovery. In practice these technologies are integrated when efforts are mounted to identify novel therapeutic treatments. For instance, the power of positional cloning techniques used in the study of family histories of disease inheritance is enhanced by the published results of the Human Genome Project, which relies on expressed sequence tag (EST) methodology. Similarly the discovery of genes coding for particular secreted proteins requires genetic mapping data and possibly computational sequence analyses. (Langer-Safer *et al*. 1997: 173-174.)

Table 5.3. Comparison of the main technologies of gene discovery.

The main gene discovery technologies				
	Expressed sequence tag (EST)	**Locating genes coding proteins secreted by cells**	**Differential display**	**Positional cloning**
The main aim of the technology	Sequencing random cDNAs from libraries of choice	Identification of genes expressing particular secreted proteins	Identification of differentially expressed genes	Locating disease-causing genes in the chromosomes of affected families
Efficiency (throughput)	High	Medium	Medium	Low
Resulting knowledge on gene function	Low	High	Medium	High
Likelihood of identifying therapeutic candidates	High (high volume but low focus)	Medium	Medium	High (high focus but low volume)
Additional work required for drug discovery	Extensive biological experimentation	Easy development if the secreted proteins are suited to therapeutic use	Discovered gene may not be the original cause but downstream	Nucleotide sequencing of the chromosome region
Future increase in productivity	High	Medium	High	Low
Cumulative results so far	Million human cDNA sequences in public databases, several million in private databases	Thousands of novel secreted proteins identified	Identification of a number of promising therapeutic targets -both genes and proteins	Explanation of dozens of inherited diseases.
Examples of results	Growth factors, erythropoetin, growth hormone and others	Osteoprotegerin (potential against osteoporosis; the gene coding leptin (related to obesity)	Identification of genes involved in atherosclerosis, bipolar disorder, metastasis	Explanation of about 35 'Finnish diseases'

Sources: Kennedy 1997, Langer-Safer *et al*. 1997, Shiue 1997.

The work to sort out the functions of genes lags behind the efforts to define partial gene sequences. There has been an intense debate on the wisdom of granting patents

to gene sequences gained by EST methods, as the functions of such sequences are often not understood. Contrary to patent claims in other fields gene sequences as such disclose relatively little novel information, but patents obtained can hamper the use of the sequences in genetic probes and DNA arrays. (Polizzi and Shetka 1997: 212-213.) The pace at which DNA chips and other technologies of functional genomics can be developed to some extent depends on the solution of patent issues (Service 1998: 397). Some of the technologies used in finding out the functions of genes are listed in Table 5.4.

Table 5.4. Comparison of methods of identifying functions of genes.

Technologies used to identify functions of genes				
	DNA microarrays	Chromato-graphy, incl. mass spectrometry	**Electro-phoresis methods**	**Antisense DNA, ribo-zymes, and knock-out mice**
Technological features	DNA oligonucleotides on semi-conductor surface	Discovery of changes in the mass of a protein expressed by mutated genes	Discovery of changes in the charges and masses of proteins	Elimination of the function of a particular gene and study of the results
Efficiency (throughput)	High	Medium	Medium	Low
Future increase in productivity	High	Medium	Medium	Low
Significance for drug discovery	Increasingly important	Well established	Well established	Growing; some ribozymes are promising thera-peutics themselves

A polymorphism can make its carrier more susceptible to some diseases, give unusual resistance to others, and even modify the efficacy of some pharmaceuticals. One example of an unusual resistance to a disease is a certain allele that obliterates the HIV-1 coreceptor on lymphoid cells and gives some resistance against HIV-1 infection. The allele may have developed approximately 700 years ago in Northern

Europe, possibly as a result of the Black Death epidemic. Individuals who inherit this mutant gene from both parents are effectively devoid of the receptor and immune to an unusual degree to the HIV-1 infection. (Stephens *et al*. 1998.)

Differences between genetic makeups of individuals can explain differences in reactions to some drugs. Only recently it was discovered that the reason why aspirin helps to protect some people from developing heart attacks is linked to the genetics of these individuals. A specific polymorphism related to their blood clotting platelets makes these people ten times more sensitive to the anticlotting effect of aspirin than individuals who do not have the polymorphism. (Cooke *et al*. 1998.) It can be anticipated that the growing awareness of polymorphisms and the massive amounts of human genomic data available in the future will lead to focused drug development efforts that will take advantage of the particular genetic dispositions of various groups of patient populations (Kennedy 1997: 112).

5.2.2 Targeting the pathogenic component of human diseases

During the last few decades the treatment of infectious diseases has relied on wide-spectrum antibiotic therapies. It has been suggested that this well-established approach may change in the long run, when pathogen-specific or at least narrow-spectrum treatments are likely to become available. The need for such a change is based on the continuing proliferation of antibiotic-resistant bacterial strains and challenges met in treating the patients suffering from immune system deficiencies. In the future new accurate and rapid diagnostic methods are likely to support the adoption of pathogen-specific treatments. Moreover, adjunctive immunotherapy strengthening the natural capabilities of the human immune system against specific pathogens may become possible in treating infectious diseases. (Casadevall 1996.)

Pathogens are factors in more diseases than was suspected only a relatively short time ago. Pathogens play a leading role in dental caries (*Streptococcus mutans*) as well as in duodenal and gastric ulcers (*Helicobacter pylori*). *Chlamydia pneumoniae* may have a causative role in cardiovascular disease (Campbell *et al*. 1998). Viruses and bacteria may also lie behind more cancers than is suspected at the moment. The World Health Organisation has estimated that a majority of some cancers are attributable to viruses, bacteria, or parasites. More than 1.5 million new cancer cases could be avoided each year by preventing the infectious disease

associated with these diseases. (WHO 1997.) In the future it may become possible to identify the role of pathogens in conditions that are initially triggered by pathogen action, but then develop very slowly. Such conditions may include some autoimmune diseases, such as asthma, rheumatoid arthritis and multiple sclerosis. Future diagnostic methods may make it possible to discover remaining DNA fragments of the pathogens involved. (Gao and Moore 1996.)

As of yet we may even not know all existing types of infectious agents. An infectious agent of a new type, consisting of misfolded variations of the prion protein, has been discovered as the cause of the bovine spongiform encephalopathy (BSE) epidemic in cattle (Liu et al. 1999). The misfolded protein is not eliminated by normal enzymes and accumulates in the brain causing a new form of Creutzfeldt-Jacob disease in humans and other fatal, but rare brain conditions. The discovery of new kinds of pathogens in the future cannot be precluded. For instance, it has been suggested that intra- and extracellular calcium phosphate deposits causing kidney stones and other medical conditions are produced by nanobacteria, that would be pathogens of a new type (Kajander and Çiftçioglu 1998). The suggestion remains controversial. Even if eventually accepted, as in the case of the prion protein, it takes years for the scientific community to ascertain the validity a hypothesis that runs counter to well-established beliefs.

As pathogen species continue their evolution, new strains are likely to emerge in due course of time. The process could be facilitated by the fact that the immune systems of millions of people have been impaired by the AIDS epidemic. Immunosuppression resulting from treatments related to organ transplants has also become more common. Impaired immune function may allow the evolution of new pathogen variants that would have been eliminated by normal immune systems. Bacteria that normally live in close association with humans and are either neutral or beneficial may be transformed to become malevolent by mutations induced by natural evolution, chemicals, pharmaceuticals or other pathogens. *Acinetobacter* and *Xanthomonas* were originally innocuous, but in a few years they evolved into multidrug-resistant strains that are capable of causing potentially fatal blood-borne infections in hospitalised patients (Levy 1998). Also it is possible that in the future more pathogens may cross over from animals to afflict humans. AIDS originated from chimpanzees and BSE-CDJ from cattle. In Central Africa there have been epidemics of the Ebola and Marienburg virus while in China there have been lethal cases of bird influenza. Lyme disease is one example of a human disease transmitted by animals in industrialised countries. In the future xenotransplants may be used to

save the lives of thousands of people, but the method also could increase the risk of introducing novel infectious agents of animal origin into the human population (Meslin 1997).

Drug resistance is a manifestation of the ability of bacteria and viruses to evolve and adapt to changing circumstances. Several bacterial strains have developed multidrug resistance. They include *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well as several strains of *Listeria* (Baquero 1997). The stockpile of efficacious antibiotics of the 'first generation' has been depleting at a faster rate than new medicines have been developed. Resistant strains have emerged and spread at a surprising speed all over the world.

Bacteria use a variety of mechanisms to fight antibiotics. Some produce enzymes that render first generation antibiotics such as penicillin harmless. Sometimes bacteria develop pumping or efflux mechanisms to remove antibiotics from the cell. Bacteria can also change the shape of molecules that are targeted by an antibiotic. (Amábile-Cuevas et al. 1995.) One of the resistance mechanisms against tetracycline is a pumping system that removes not only tetracycline but also other antibiotics from the bacterial cell. In another method the tetracycline target, protein-producing ribosome, is modified and the structure on which the drug binds is changed. The third mechanism involves the modification of the tetracycline compound itself, but it has been discovered only in two anaerobic bacterial strains. (Schnappinger and Hillen 1996.)

Genetic information coding the resistance mechanisms is usually stored on plasmids, tiny loops of DNA. Many plasmids have originally evolved to help bacteria survive hazards in the environment. Plasmids are relatively easily transmitted between individual bacteria of the same species and even between individuals of different species. Resistance genes can also be transferred by viruses from one bacterial cell to another. Occasionally, a bacterium can also acquire a gene from the remains of a dead bacterial cell.

Compounds that would neutralise resistance mechanisms would be useful in developing 'antibiotics of the second generation' or adjuncts that would enhance the efficacy of existing antibiotics. For instance, the efflux pump would be a tempting target for countermeasures against multidrug-resistant bacteria. Of the known efflux mechanisms one of the most efficient is that based on the multidrug transporter

protein MdfA. By using a single negatively charged amino acid residue the transporter protein is able to identify a number of antibiotic compounds and remove them from the bacterial cell. (Edgar and Bibi 1999.) However, as the various resistance mechanisms are based on systems that have emerged in natural evolution, they are likely to be well protected against interference. The ability of these systems to evolve is already proven by the drug resistance itself. That is why the search for antibiotics of the second generation is not likely to be easy.

Usually investments in research and development expand technical opportunities and create opportunities for creating new products. However, new technologies can have negative effects as well, because they can reduce the potential for innovation in neighbouring fields of technology. In biotechnology bacterial resistance due to the use of antibiotics as growth-promoting factors in animal husbandry may eliminate opportunities that could be used to develop pharmaceuticals for humans. A case in point is everninomicin, which has shown some promise as a potential new antibiotic; it contains compounds that have not been applied in any class of medicine used in people. However, everninomicin resembles another oligosaccharide, avilamycin, that has been used in animal husbandry for several years. In a recent study it was found that, as a result of the use of avilamycin, there already are bacteria whose susceptibility to everninomicin has declined even before the human use of the antibiotic has started. (Aarestrup 1998.) It is evident that antibiotic resistance phenomena can significantly increase the costs of antibiotic drug research and reduce the profitability of future drug development efforts.

Outer membrane proteins of several pathogens are obvious targets for future drug development efforts, as some of the most successful antibiotics, including penicillin and vancomycin, impede the proper synthesis of the bacterial cell wall. Chlamydial infections are common, and the major outer membrane protein of Chlamydia trachomatis is of great interest in vaccine development. However this protein exhibits considerable antigenic variation, as do many other focii of vaccine research (Brunham and Peeling 1994). The cell envelope of *Mycobacterium tuberculosis* contains several unique polymers and the inhibition of their biosynthesis is one aim in efforts to develop narrow-spectrum pharmaceuticals for the treatment of tuberculosis (Andersen 1997).

Some bacterial species communicate. They send chemical messages by secreting messenger compounds. A common household bacterium *Pseudomonas aeruginosa*, responsible for many hospital infections and a leading cause of death in cystic

fibrosis patients, resists antibiotics by forming a sticky protective biofilm. Its formation is triggered by a homoserine lactone chemical that the bacterium releases. When there is enough of the chemical in the environment, the production of biofilm is started. A gene controlling the process has been discovered. Possible targets for future antimicrobial compounds include the messenger chemical, the gene controlling biofilm formation, and intermediate steps in the process. When the formation of biofilm is prevented, *Pseudomonas* is left almost defenceless against antibiotics. (Davies *et al*. 1998.)

The development of antiviral drugs and vaccines against human immunodeficiency virus (HIV) and hepatitis C virus (HCV) has proved very difficult, as these viruses mutate constantly. Each HIV virion carries two complete genomic strands based on RNA. Natural recombination of these strands is possible when a single cell is infected with two different strains. Resulting recombinant HIV strains have been found in patients in regions where multiple HIV variants circulate. Variations arising from recombination poses difficult problems for the development of HIV drugs and vaccines. (Burke 1997.)

In the future it may become possible to design therapeutics that would attack specific vital, slowly mutating RNA sections of the HIV. Therapeutics focusing on RNA could utilise ribozymes, enzymes that can be modified to bind with any part of the RNA molecule. Ribozymes could be administered as drugs or, in a more distant future, a gene therapy could be used to insert a ribozyme-encoding gene into host cells. Added protection against the emergence resistant strains could be achieved by targeting multiple sequences of the viral RNA. (Welch *et al*. 1998.) In experimental studies with HIV success in the form of very high inhibition rates of viral progeny production has been reported (Menke and Hobom 1997).

Immunisation is the most cost-effective way of dealing with pathogens, and in the future DNA vaccines are likely to be developed against a number of human diseases. The mechanism used in DNA vaccines resembles viral infection. The aim is to use the biosynthetic machinery of the host cell to synthesise a foreign protein. The protein then becomes an antigen to which the immune system reacts resulting in immunity. Plasmid DNA coding a target protein, for instance a protein found on the surface envelope of a pathogen, is introduced into the host organism by injection or particle bombardment. A small number of host organism cells absorb the DNA code and express the protein. The host immune system then reacts to the protein and the result is protection against the pathogen as the immune system will be able to

recognise the antigen protein in the future and will produce antibodies rapidly. It may become possible to develop DNA vaccines that contain not just one but even thousands of DNA strings expressing proteins of a particular pathogen. Such an approach might be used in future emergencies, when a vaccine is urgently required against a new virulent pathogen. (Whalen 1996.)

DNA vaccines are evolving into a versatile methodology. Trials of an HIV vaccine were started in 1999 in Finland. Elsewhere research is being carried out on the use of DNA vaccines in inhibiting symptoms of allergic asthma as well as conferring protection against Hepatitis B disease and malaria. Another vaccine is being developed against tick-born encephalitis caused by flavivirus. In many of the studies the DNA vaccine is being administered together with an immune system adjuvant in order to elicit a stronger immune system reaction and better protection. DNA immunisation has also been used to prevent or inhibit tumour development. (Lai and Bennett 1998; Butts *et al.* 1998.) Clinical trials have been carried out on patients with lymphoma and metastatic melanoma (Whalen 1999).

5.2.3 Pharmaceuticals targeting cancer cells

Tumorigenesis can be best characterised as a process caused by five or six accumulating genetic alterations. Mutations in oncogenes promote proliferation of cancer cells, while changes in genes governing programmed cell death (apoptosis) inhibit the onset of a natural cell elimination process. Mutations in apoptosis genes such as P53 and in oncogenes such K-ras are often implicated in studies of tumorigenesis. In the future DNA tests may be used in screening large populations for cancers enabling early detection and treatment. After diagnosis the genetic profile of a patient's primary tumour could be used to define prognosis and optimise treatment. (Caldas 1998.)

Most present-day cancer treatments involve the use of relatively blunt methods such as surgery, poisonous chemicals and radiation. In the future it may become possible to develop increasingly smart methodologies to diagnose not only the type of cancer, but also its stage and characteristics. As all cancers are genetically unstable, lots of genetic variation and mutations can be expected to occur between various stages and even regions of a single tumour. That is why treatments relying on a single antibody or a single DNA target are not likely to be very successful.

Gene therapy relying on several genes may be a part of the solution. Over the last few years more than one hundred clinical trials involving the use of gene therapy in cancer treatment have been approved in the world. Viral vectors that have been tested include retroviruses and adenoviruses. Nonviral methods include liposomes and direct injection. In targeted delivery tumour- and tissue-specific antigens have been the selected binding sites. Therapeutic genes used in the trials have included cell suicide genes, genes preventing the functioning of oncogenes (based on antisense technology and ribozymes) and genes coding enzymes that enhance the efficacy of cancer drugs. (Dachs *et al*. 1997.)

Treatment of many cancers is complicated by the physical structure of solid tumours. Cancer cells degenerate rapidly, and solid tumours always have some dead tissue that protects the tumour against the penetration of drugs and vectors carrying therapeutic genes. Solid tumours also hamper adequate blood supply to adjacent healthy tissues, and as a result neighbouring cells as well as cancer cells themselves often suffer from lack of oxygen. Hypoxic cells are usually resistant to radiotherapy and chemotherapy. However it may become possible to use hypoxia response elements as triggers in activating therapeutic genes in healthy tissues adjacent to the tumour. (Dachs *et al. ibid*.)

Rapidly growing tumour cells fight for oxygen by expanding the network of blood vessels feeding them. It may become possible to prevent the growth of vasculature serving the tumour cells, and to destroy selectively these blood vessels, thus starving and suffocating the cancer to death. Promising results have been achieved in animal trials in which antiangiogenic factors have been tested in restricting blood supply to tumours. Tumour blood vessels can be selected for drug targets, because the endothelial cells of rapidly growing blood vessels contain growth factors that are lacking from normal veins. (Arap *et al*. 1998; Ruoslahti 1998.) Vascular endothelial growth factor seems to be elevated in many cancer patients regardless of the histological type of the cancer. (Salven *et al*. 1997.)

In some cases the human immune system is able to eliminate cancer cells before they become numerous enough to be a threat. Indirect evidence is presented by a particular immunity mechanism, that has serious side effects. About one in every 1000 women having breast or ovarian cancer develops a protective immunity, in which the immune system targets a cancer cell protein known as *cdr2*. Unfortunately the same protein can also be found in the cerebellum area of the brain. Consequently the immune response also affects the brain, and these women

develop a neurological condition known as paraneoplastic cerebellar disorder that is usually diagnosed before the cancer. (Albert *et al*. 1998b.) Cytotoxic T lymphocytes (CTLs) play an important role in immunity and resistance against cancer. Antigens released by dying cancer cells are carried by the blood stream to dendritic cells, which then alert CTLs to attack cancer cells exhibiting the antigens. (Albert *et al*. 1998a.) Animals studies have shown some success in fighting cancer by vaccinating animals with dendritic cells that have been made to carry tumor antigens (Gilboa *et al*. 1998).

In the future it may become possible to mobilise the strengths of the human immune system against cancer cells of various types. Particular groups of people, identified in population screenings as having a high risk of developing a cancer of a certain type, could be offered some protection with the help of DNA vaccines targeting some features of cancer cells. Moreover, it may become possible to define mechanisms that protect cancer cells from being discovered by the immune system (Zheng *et al*. 1998). If it became possible to turn off the protection, the immune system would be able to destroy the malignant cells.

5.2.4 Pharmaceuticals related to human and pathogen enzymes

For the pharmaceutical industry human and pathogen enzymes are of immense importance. Aspirin, an analgesic that has dominated the market for a century, blocks the production of the cyclo-oxygenase (COX) enzyme thus inhibiting the synthesis of prostaglandins. These fatty acids help the body to produce symptoms of pain and inflammation. Unfortunately the human stomach lining is protected by the COX enzyme and its inhibition easily leads to stomach ulcers. The discovery of the COX-2 enzyme provided the drug industry with a new target that helps to avoid gastrotoxic effects. (Hawkey 1999.) The annual world market for the new COX-2 inhibitors is estimated to amount to $5 billion (Pilling 1999).

The influenza virus uses an enzyme called neuraminidase to free itself from the human cell where it was synthesised. Recently neuraminidase inhibitors have been developed, and it has been suggested that inhaling these medicines daily during an influenza outbreak could reduce the chance of catching the disease. The drug appeared to shorten bouts of flu by about 2 days in trials in Europe and Australia,

but a similar study among U.S. patients produced lesser results. (Read 1998; AP 1999.)

Enzymes repairing DNA are of great interest to the pharmaceutical industry. They sustain life, protect against cancer, remedy damages caused by ultraviolet (UV) light and may prevent some of the biological changes associated with ageing. Treatments based on DNA-repairing enzymes could be particularly useful in preventing skin cancer, one of the most rapidly increasing forms of cancer. In humans an enzyme called glutathione peroxidase counteracts harmful effects of UV radiation. It seems that a virus causing a skin disease has been able to acquire the gene coding the enzyme; as a result the virus has gained some protection against UV radiation. (Shisler *et al*. 1998.) Recently it has been shown, that an error-prone repair enzyme may be responsible for much of DNA damage associated with ultraviolet light (Tang *et al*. 1999).

An enzyme called telomerase, not found in normal cells, repairs telomeres, specialised nucleic acid structures located at both ends of linear eukaryotic chromosomes. Each time a cell divides, telomeres get shortened, and finally, when telomeres get very short, cell division is prevented. The mechanism may act as a molecular clock marking and controlling ageing processes. In some cancer cells telomeres are maintained by telomerase activity, enabling uncontrolled growth. (Shore 1997: 233.) In the future telomerase may be used to counteract some features of the ageing process. Similarly substances inhibiting telomerase in cancer cells may be used in the development of new cancer treatments. (Klingelhutz 1997.)

A special case of the power of DNA repairing enzymes is *Deinococcus radiodurans*, a microbe discovered in 1956 in a can of meat that had been radiated in order to sterilise it. The microbe not only endures acute radiation doses of up to three million rads, but it grows even when exposed to radiation continuously. The bacterium is able to repair damage to its DNA efficiently. The trait probably evolved as a response to extreme droughts that damaged the bacterium's DNA. (Lange *et al*. 1998.) DNA polymerases carry out a wide variety of synthetic tasks during DNA replication and repair. These enzymes range from variants that deal with single nucleotide gaps to enzymes repairing gaps of up to kilobase size. (Burgers 1998.) Ribozymes are special enzymes capable of cleaving and repairing RNA at specific sites. It is possible that in the future combinations of new approaches such as gene therapy, ribozymes and immunotherapies will be used simultaneously to inhibit multiple stages of the life cycles of infectious agents (Bunnell and Morgan 1998).

Enzymes called phosphoinositide kinases are located on the cell membranes of most types of cells; they receive and relay chemical signals. Some types of disorders seem to be related to the signalling system, causing cells to proliferate and travel to distant parts of the body, leading to cancers. The signalling system is also important for nerve cells. In a post-mortem study of depressed suicide victims the phosphoinositide system was found to be impaired by about 30 % compared to the normal level. (Pacheco *et al*. 1996.)

5.2.5 Pharmaceuticals for the central nervous system

We may recall how attempts to explain gastric ulcers on the basis of personality and behaviours of patients were not very useful in developing medical treatments to the condition. Similarly efforts to explain medical conditions related to the central nervous system on the basis of personality traits, personal experiences or behaviours have not been very useful in the development of efficacious pharmaceuticals. Instead, psychopharmaceutical drug leads have been often discovered on the basis of random events. The value of monoamine oxidase inhibitors in treating depression was discovered in the 1950s as a result of a trial of a drug that was designed for the treatment of tuberculosis. The efficacy of lithium in dampening both the manic and depressive phases of the bipolar disorder has been known for half a century, but a comprehensive explanation was found only recently. It seems that lithium stabilises glutamate uptake in presynaptic nerve endings in the cerebral cortex (Dixon and Hokin 1998). Glutamate is an important excitatory neurotransmitter found in most regions of the brain.

The discovery of neurotransmitters and their receptors put the psychopharmacological drug discovery process on a rational footing. The effects of chemicals that relay signals between neurons can be amplified, reduced, mimicked and blocked. As a result new pharmaceuticals have been developed, and researchers have improved their understanding on how biomolecular processes in the central nervous system are related to mood disorders and addictions. It has also been shown, how abuse or neglect of young mammals and humans may lead to biomolecular changes in the central nervous system. Restoring the biomolecular balance could help these individuals to overcome the effects of past events.

The regulation of neurotransmitters such as serotonin, dopamine, norepinephrine and acetylcholine is the aim of many on-going drug research efforts. Each of these research areas is promising in itself, but the central nervous system is a complex environment, in which neurotransmitters and receptors have several variants and different functions in separate circuits (Greenfield 1998). A pharmaceutical relating to a single transmitter may provide a step into the right direction, but may not be enough to restore the overall balance of the complex system. Therefore it is likely that cocktails of transmitter-related medicaments will be designed in the future for the treatment of various subtypes of mood and addictive disorders. Restoring the balance between several transmitter systems may be the key in the treatment of, for instance, panic disorder (Coplan and Lydiard 1998).

It is likely that pharmaceuticals will be developed in the future for the treatment of some forms of obesity and substance abuse. Some of the dopamine receptors of the brain are involved in addictive behaviours (Tiihonen *et al*. 1995), indicating that medications facilitating changes in these behaviours may be possible in the future. Similarly, if sensory reactions caused by food are particularly strong among some obese people (Karhunen 1998), in the future these reactions may be alleviated with appropriate medication. Antiobesity agents in clinical trials include leptin, a compound discovered in 1994. High leptin levels seem to signal to the central nervous system that energy reserves are sufficient, whereas low levels signal the need for increased energy intake (Auwerx and Staels 1998).

Molecular mechanisms of several neurodegenerative diseases have been discovered in the 1990s. The recipe for success has been straightforward: "...identify pathogenic genes by positional cloning, by cloning genes that encode proteins involved in the disease, or by combining the two approaches; find pathogenic mutations; and then model and study the disease in cells by transfection and in mice by transgenesis." (Hardy and Gwinn-Hardy 1998: 1075.) As the causes of the diseases are now better understood, new subtypes have been discovered, and the classification principles of the diseases can be redefined. Mutations in the so-called tau protein seem to lead to most cases of Parkinson's disease, while alterations in the α-synuclein protein are the main cause of Alzheimer's disease. (Hardy and Gwinn-Hardy *ibid*: 1078.) In the future early diagnosis methods may become available enabling pharmaceutical therapies that will at least delay the onset of typical symptoms.

Efforts to develop drugs against neurodegenerative diseases may lead to the emergence of compounds that have some relevance in enhancing individuals' capacity to learn, remember, and recover mentally after stress. Cholinergic and glutamatergic neurotransmission mechanisms participate in learning and memory processes, and they are important target areas in research on pharmaceutical leads that would alleviate the symptoms of Alzheimer's disease. Similar compounds could be beneficial also for children having certain types of learning disabilities. (Capone 1998.)

It is likely that mental conditions such as schizophrenia comprise a great number of variants and causes. Schizophrenic symptoms arising in adolescence or adulthood can be a result of perturbations that have taken place in corticolimbic circuitry during prenatal development or in early childhood (Benes 1993). New diagnostic tools would be urgently required to enable accurate diagnosis of subtle variants of schizophrenia and other conditions affecting the central nervous system. In the case of depression, the level of corticotropin-releasing factor (CRF) would seem to be an important target for measurements. Some cases of mental disorders are caused by infectious agents such as herpes virus and Borna disease virus. (Dietrich *et al*. 1998.) Accurate diagnostic tools of the future may create an improved basis for the treatment of various subtypes of medical conditions involving the central nervous system.

5.2.6 New drug manufacturing technologies

Biotechnology creates methods that complement traditional techniques of pharmaceutical production and others that can revolutionise production techniques and reduce manufacturing costs significantly.

Chiral compounds produced by using biocatalysts provide an example of the use of biotechnology in enhancing traditional production techniques. All proteins in the body are stereospecific; that is, they can react differently to the two chiral forms of molecules. One chiral form or enantiomer of thalidomide is a useful pharmaceutical, while the other causes birth defects. In the early 1990s there were about 500 synthetic chiral drugs on the market, but about 90 % of them were sold in a form that included both enantiomers. (Samdani 1993.) Since then pharmaceutical companies have made great efforts to replace the mixtures with single enantiomers.

By using enzymes drug manufacturers can avoid the production of an unwanted enantiomer and reduce harmful side-effects.

Biotechnology enables the recruitment of bacteria, plants and animals for pharmaceutical production. Procaryotic cells (bacteria) or eucariotic cells (yeasts, animals) can be used in the production of proteins of therapeutic interest, but not all cell types can be harnessed to produce any protein. For instance, bacteria cannot produce glycoproteins, since they lack the capacity to glycosylate (Kadir 1997: 53). The use of Gram-negative bacteria is hampered by toxic lipopolysaccharide contained in their outer membranes that are more complex than those of Gram-positive species (Hoekstra and Smeekens 1997: 2-3).

Among plants tobacco is a favoured species used as a host for genes coding pharmaceutical proteins. Tobacco is relatively easy to modify, grows rapidly, and produces large amounts of seed, facilitating production increases when necessary. Transgenic plants seem to be particularly suited for the production of fully functional antibodies, including complicated and expensive secreted IgA antibodies for anti-inflammatory therapeutics. (Junzhi *et al*. 1999.)

The problems of using transgenic plants in pharmaceutical production include low yields and difficulties in extraction and purification. A remedy is to make plants secrete the desired proteins so that they can be harvested continuously. Tobacco plant roots have already been experimentally engineered to secrete xylanase enzyme into a hydroponic medium from which the enzyme can be easily extracted (Borisjuk *et al*. 1999). The ability of roots to secrete proteins originally evolved to protect the plants against bacteria and other pathogens. Another secretion pathway that may be exploited in the future is leaf guttation, commonly known as morning dew.

Even though transgenic plants can be made to produce many human proteins, the plants are not always able to carry out the necessary postsynthetic modifications required to make these proteins fully functional. In transgenic animals the modifications are often performed as a matter of course. That is why transgenic animals are likely to be a preferred source of many pharmaceutical proteins, such as hormones, enzymes and vaccine proteins. (Jänne *et al*. 1998.) In the future human lactoferrin for the treatment of gastrointestinal infections as well as various expensive blood clotting factors are likely to be produced at a relatively low cost in the milk of transgenic cows, pigs or goats.

5.2.7 New drug delivery techniques

In drug development projects problems related to pharmacokinetics and drug delivery often cause considerable costs and risks. Methods that can be used to discover and solve potential problems at an early stage of drug development would be important for reducing the costs of drug development.

Drug delivery to the central nervous system is a particularly tough challenge. The blood-brain barrier consists of unusually tight capillary walls; their protective effect is enhanced by glial cells wrapped around them. The barrier prevents the entrance of large molecules, including proteins, biopharmaceuticals and water-soluble chemicals. Among the few peptides passing through the barrier are insulin and leptin, and most of the lipid-soluble molecules can also pass through. Injectable biodegradable microcapsules and microparticles releasing drug compounds in a controlled manner as well as collagen gels are some of the options for reaching the central nervous system. In the long term it may become possible to engineer and inject modified stem cells that produce the desired protein within the central nervous system. Such cells could offer the additional benefit of replacing dysfunctional neurons, thus strengthening weakened brain functions. (Maysinger and Morinville 1997: 417.)

New general drug delivery new methods such as transdermal and nasal routes as well as aerosols penetrating deep into lungs are likely to be used increasingly in the future. From the point of view of biopharmaceuticals pulmonary delivery is an interesting option, as the route is likely to prove particularly suitable for the delivery of peptides and protein-based compounds, including insulin (McCarren 1998). In the future transgenic pharmaceutical production technologies may be combined with genes that code structures that give pharmacokinetic protection to the drug compounds.

Encapsulation in polylactic acid materials has already been approved for use in controlled drug delivery. In a method patented in the United States, drugs can be incorporated into macrophage cells. The cells can then be injected intravenously, and the final phases of delivery take advantage of the homing mechanism of the macrophages. (Yatvin *et al*. 1998.) Similarly a method for the encapsulation of non-

diffusible drugs into human red cells has been developed. The resulting processed erythrocytes are expected to survive normally *in vivo*. (Magnani *et al*. 1998.)

In the future it may become feasible to use antibodies to carry pharmaceutical compounds or radionucleids directly to target cells in the body. Antibodies bind only to specific molecular sites of pathogen or cancer cells; in addition human antibodies also have a long half-life. Recently it has been established that alpha-radiation emitting bismuth-213 can be attached to monoclonal antibodies of leukemia cells produced by mice. After being injected the antibodies carry the radiating substance directly and accurately to leukemia cells, which will be then destroyed by the radiation. Promising clinical trials have been carried out with human leukemia patients in the United States. (Nikula 1998.) One of the main problems of antibodies is the rapid rate of pathogen and cancer cell mutation.

As electronic and sensor systems are being miniaturised, implantable pumps may become feasible in the delivery of medications for chronic conditions such as diabetes. Feasible mechanisms for controlling the level of medication include timed control, osmosis, programmed control, and self-regulating closed-loop control. Refills could given in coded pharmaceutical cartridges that would be automatically recognised by the devices in order to eliminate the possibility of user errors. (Bremer *et al*. 1997.)

5.3 Conclusions

The present assortment of pharmaceuticals is based on the relatively low numbers of molecular targets and pharmaceutical compounds. Over the last few years the number of new molecular entities approved for pharmaceutical use has been between 35 and 50 per annum. Developing and launching new drugs takes 10 to 15 years, and that is why one of the special features of the pharmaceutical industry is the need for longer time horizons than in other areas of high technology.

In the short run there is going to be a deluge of new pharmaceutical lead compounds and targets. The main factor is the Human Genome Project, which is expected to produce an accurate sequence of the human genome by the year 2003. At the same time the elucidation of the functions of human, animal, plant and microbial genes is expected to proceed at a rapid pace. The methods of combinatorial chemistry, high throughput screening and computerised molecular modelling will also contribute,

and as described above, new targets for pharmaceutical intervention are likely to emerge in such areas as pathogens, cancer diseases, enzyme deficiencies, and medical conditions of the central nervous system. Moreover, drug production and delivery technologies are also going to advance. The question is, at what pace can the new leads and targets be taken advantage of by developing fully functional, clinically tested pharmaceutical products.

In the medium run advances in methodologies of drug development can be expected to speed up not only the discovery of new drug leads, but also the pharmacological design as well as preclinical tests of new pharmaceuticals. Yet there is a risk that the potency of available medicines against microbial infections will continue to fall. Another risk in the medium run is that the costs of pharmaceutical research will remain high; that is to say that productivity in pharmaceutical research will continue to be low when measured in terms of new molecular entities approved for pharmaceutical use. Both risks are significantly reduced, but perhaps not completely eliminated, by the rapid advances that can be expected to be achieved in the methods used in pharmaceutical R&D.

In the long run the methodologies of the present biotechnical paradigm will be revised and new methods will be developed for pharmaceutical research. Perhaps it will become possible to develop automated multistep systems which will combine high throughput systems and other processes. Or perhaps the integration can be realised by setting up computerised systems that rely on molecular databases on lead compounds and are capable of simulating chemical reactions in the human body. What is certain is that there will be great advances both in bioinformatics and in research laboratory automation, and that the pharmaceutical research frontier will advance into yet more challenging depths and complexities.

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Technological opportunities in biotechnology

This report presents a survey of technological opportunities in a few particular fields of biotechnology: biocatalyst technology, biomaterials, medical diagnostics diagnostics, and pharmaceuticals. The map of technological opportunities has been created on the basis of recent international research findings.