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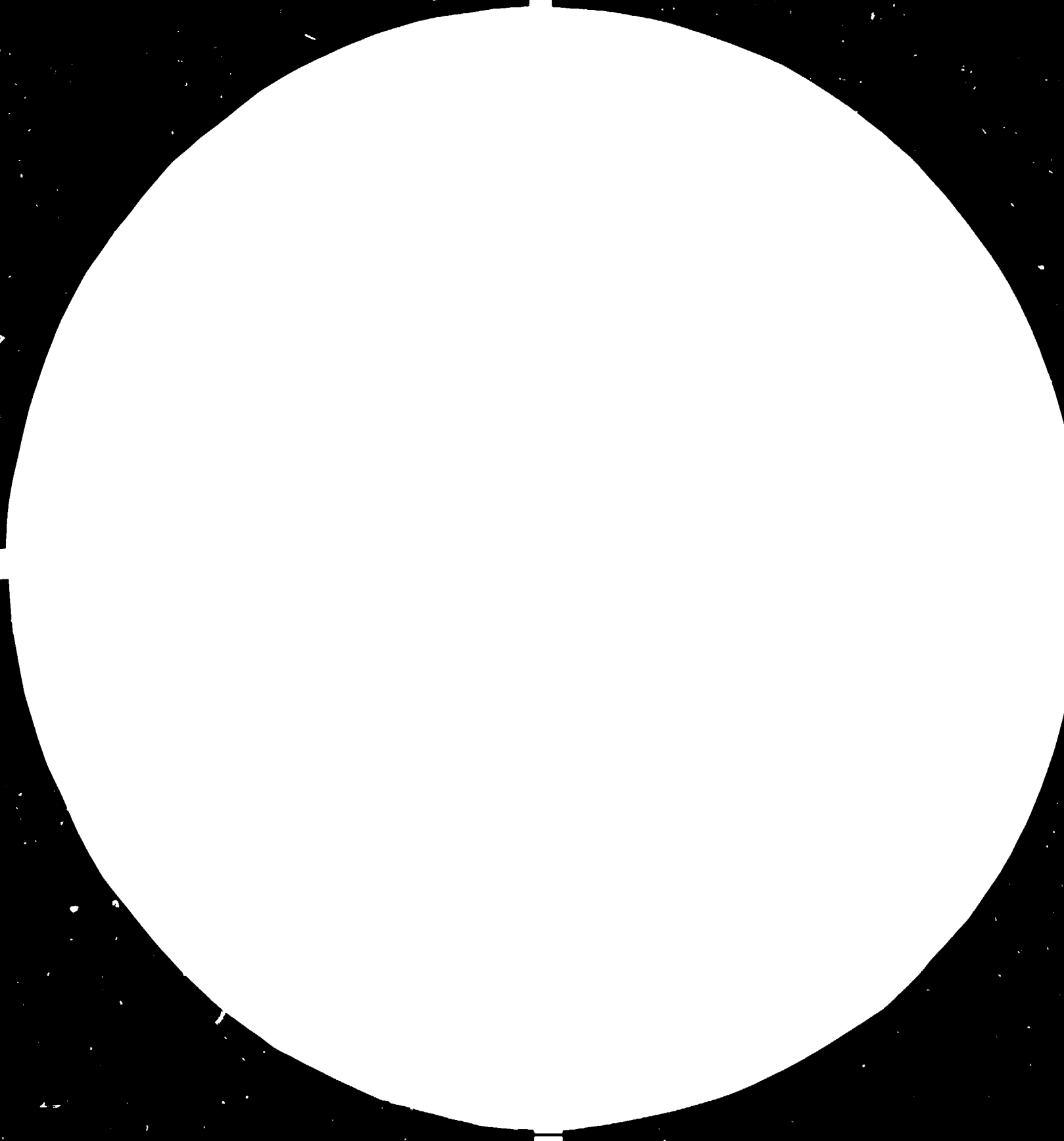

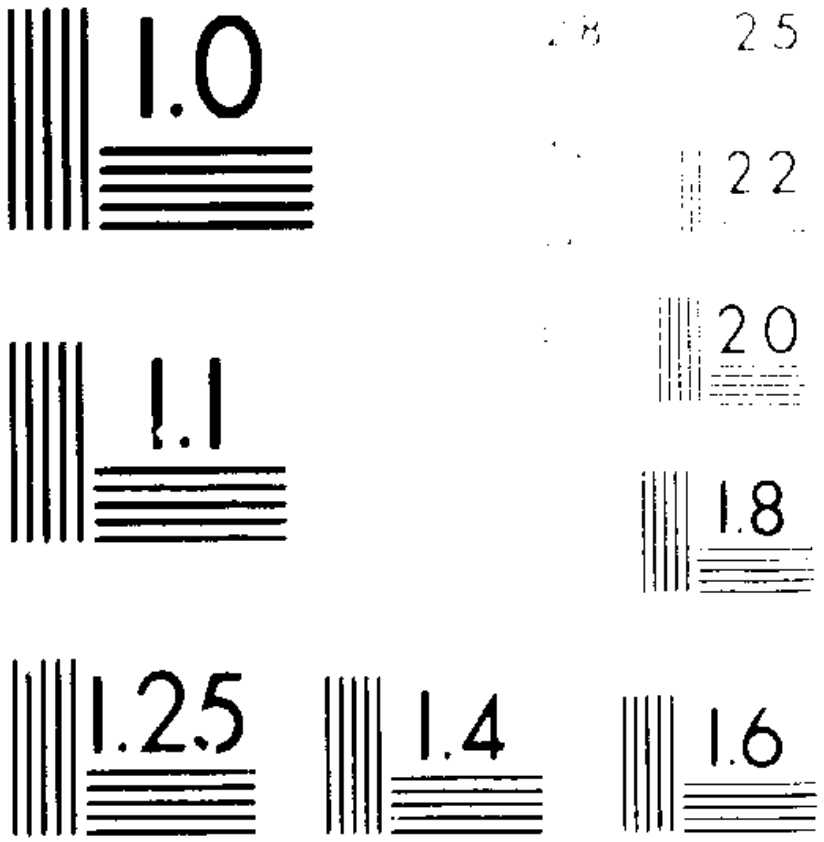
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APPLICATION OF GENETIC ENGINEERING TO THE PHARMACEUTICAL INDUSTRY

by

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1983

Introduction

Genetic engineering techniques are now widely used in modern research laboratories which are devoted to molecular biology, microbiology, immunology, genetics etc. and methods for establishing bacterial strains which contain and express genes from higher organisms are today regarded as routine procedures. Hundreds of genes from humans, animals, plants, yeast, bacteria etc. have been cloned by the use of bacterial vectors and their structure are in many cases precisely known at the molecular level. The impact of the recent advances in molecular cloning techniques is enormous and industry based on modern DNA technology is today an extremely rapidly growing branch. Several problems remain however yet to be solved, particularly those concerning efficient expression of modified proteins. Also general techniques for identification of specific genes need still to be improved.

The most obvious industrial application of genetic engineering today is within the pharmaceutical industry. Many practical applications are already a fact and gene technology will undoubtedly play a major future role in this industrial sector. Genetic engineering could be utilized in the pharmaceutical industry in several different ways and some of the more obvious examples are given below:

A) For production of antigens for vaccine purposes.

B) For production of peptides and proteins to be used as drugs in human and veterinary medicine.

C) For production of enzymes to be used for the biosynthesis and/or modification of drugs.

D) For protein engineering.

E) As a tool in Cell biology, i.e. in basic research to search for new pharmaceuticals to be used in human and veterinary medicine.

F) Cell technology.

G) For application of recombinant DNA-technology to microbial genetics; i.e. to tailor new pharmaceuticals which inhibit the replication of microorganisms.

H) To use recombinant DNA technology for diganostics of genetic diseases and for identification of microorganisms.

Infectious diseases play a paramount role in the developing world. Therefore the application of genetic engineering for vaccine production will be discussed first. Most technical aspects which are considered in this section are applicable to the other sections of this report.

A:1) The potential use of recombinant DNA technology for production of human and animal vaccines

Techniques for production of efficient vaccines have been known for more than a century and it is today a well known fact that smallpox has been eradicated from the earth due to a successful vaccination program. Also the poliomyelitis vaccination program has been of tremendous importance to mankind. The classical methods for vaccine production are however in many respects cumbersome and expensive since the production of animal virus vaccines requires large scale growth of tissue culture cells. Some microorganisms which are of great medical significance are, moreover, difficult to propagate under laboratory conditions. It is anticipated that genetic engineering could circumvent these shortcomings and be used to produce efficient vaccines.

Vaccines prepared by recombinant DNA technology, although not yet used in practice would be expected to have a number of advantages as compared to vaccines produced by conventional methods:

1) The vaccine will not contain the entire infectious agent, thus eliminating the risk of spreading infections during the manufacturing process or by the handling of large volumes of the infectious agent. This problem is of considerable importance, for instance, in connection with production of vaccines against hepatitis B virus and foot and mouth disease virus.
2) No inactivating processes are required and loss of activity due to deleterius effect of the inactivating processes is avoided.
3) No test of innocuity of the vaccine is required at least in terms of residual infectivity.
4) Techniqually demanding large scale cultivation of mammalian cells is not required.

5) Considerably larger quantities of any vaccine could be produced by recombinant DNA technology. Hence, shortcomings in potency may be overcome by scale of procution.
6) Handling of the vaccine will be less demanding since vaccines can most likely be produced which do not require refrigeration and the same careful handling as conventional vaccines. This will facilitate storage and distribution.
7) Production costs are likely to be cheaper and the vaccine supply will essentially be unlimited, once a suitable recombinant strain has been constructed for vaccine production.

To this list of advantages can also be added the fact that recombinant DNA technology will allow production of vaccines against microorganisms which are difficult, dangerous, or impossible to propagate in the laboratory. Important human diseases like hepatitis and malaria belong to this category.

The strategy which needs to be adopted will differ depending on the microorganisms against which the vaccine is to be prepared. The causes of human and animal infectious disease are bacterial, viral as well as protozoological pathogens. All three categories of pathogenes are of considerable importance although it should be noticed that genetic engineering is more easily applicable and potentially advantageous in the manipulation of viruses as compared with bacteria or protozoa.

The principle behind the use of genetic engineering for vaccine production is that one or a limited number of genes from the pathogen in question are inserted into a vector which then is transferred to a suitable host for expression. A prerequisite for the construction of vaccine producing strains is that the gene is known that encodes the antigen or the antigens which induce protecting antibodies. The protecting antigen is known for some viruses and it is then a comparatively simple task to identify the appropriate gene since most viruses contain a small amount of genetic information. For the more complex pathogenes this is not the case and considerable amounts of basic research will be needed to identify the appropriate antigen and then to isolate the corresponding gene in order to have it expressed in a recipient cell. Among the problems which require additional work before recombinant DNA technology becomes a general method for vaccine production should be mentioned:

1) Molecular studies of gene structure and regulation in the more complex pathogens.
2) Identification of antigen(s) which give rise to protecting antibody.
3) Expression in the recipient of antigens which are active with regard to antibody induction. This will sometimes require modification of the protein and/or cleavage.
4) Purification of the appropriate antigen. This is an important problem since vast quantities of antigen will be needed in many cases.
5) Development of stable antigen preparations which give rise to a strong and long lasting antibody response.
6) Problems associated with antigen variation.

A:2) The present international status of ongoing research on vaccine production

The potential use of recombinant DNA technology for vaccine production was recognized a long time ago and several laboratories are already engaged in the development of vaccines against a few pathogenes. Among the ongoing projects should be mentioned the production of a vaccine against foot and mouth disease virus (FMDV). The gene which elicits neutralizing antibody against FMDV has been cloned and successfully expressed. A vaccine trial including a small number of cattle has given promising results and the problems associated with the application of recombinant DNA technology to FMDV vaccine production appear to be solved in principle. What remains to achieve are the construction of multivalent vaccines, to optimize expression, to develop simple purification procedures and to prepare potent antigen preparations. It seems, however, likely that recombinat DNA technology will play an important role for the solution of the FMDV problem.

Another important medical problem which has been approached through genetic engineering during recent years is hepatitis. The genome of hepatitis B-virus has been cloned and its structure is known at the nucleotide level although the virus cannot be propagated in tissue culture cells. The surface antigen which is needed for vaccine production provides a more difficult problem to

The most promising aspect of the industrial application of genetic engineering today is within the pharmaceutical industry. Many practical applications are already the fact and the new DNA technology will undoubtedly play the major role in this industrial sector. Genetic engineering could be utilized in industry in many different ways as listed below.

1) The most obvious use of recombinant DNA technology is for the production of peptides or proteins of medical importance. This can be divided into a) production of antigens for vaccine purposes.

A:3) Future prospects for vaccine production by genetic engineering

It should be pointed out that vaccine production by genetic engineering is not always the method of choice for combatting infectious disease. Potent vaccines are in some cases already produced by conventional procedures and genetic engineering does not always offer any obvious advantage. It may furthermore be extremely difficult in some cases to construct vaccines against the more complex pathogens, particularly in cases when frequent antigen variation occurs. Therefore chemotherapy must always be considered as an alternative to vaccine production. A vaccine program will require substantial amounts of basic research in order to elucidate the molecular biology and the metabolism of the more complex pathogens and it is hence desirable that such a program is intimately coordinated with a program for chemotherapy against tropical disease.

A general outline for the construction of human and animal vaccines by genetic engineering is given below.

A:3.1) Production of vaccines against small non-enveloped viruses like FMDV.

A successful solution to the problem of FMDV vaccine production has already been offered. The principle behind the approach is rather simple, since the gene encoding the protecting antigen has been identified. DNA fragments or rather cDNA copies of a viral RNA are inserted into a bacterial vector and expression is achieved by using signals already existing in the vector. The result will in most cases be a fused protein consisting of bacterial and viral sequences. This particular property does not seem

to be a problem but could rather be an advantage for the purification of the antigen. Although bacterial strains have already been constructed which produce FMDV antigen that is suitable for vaccine production, optimization will be needed since massive quantities of the vaccine will be required in order to carry out a successful eradication program. Thus, alternative host/vector systems which are easier to handle, cheaper to grow and which export the antigen into the growth medium will have to be designed. Finally, the immunogenicity of the antigen must be evaluated carefully and preparations will have to be designed which provide long lasting protection. The latter requirement may involve sophisticated manipulation of the gene encoding the antigen so that an altered protein is produced which is a more potent immunogen than the natural antigen.

A3:2) More complex viruses with modified antigens (rabies, influenza hepatitis etc.)

The basic strategy is the same as described in the previous section. In cases where the protecting antigen is known the appropriate DNA fragment carrying the gene or a cDNA copy of a viral RNA is inserted into a suitable vector in order to obtain expression. Successful expression is monitored by immunoprecipitation. Special care will have to be taken if the antigen is modified by glycosylation or cleavage. In that case the role of the modification on the induction of protecting antibodies will have to be properly evaluated. If the modification is of importance a more complicated strategy must be adopted, involving eucaryotic host/vector systems.

The problem of antigen variation must also be considered. Field studies will in some cases have to be carried out in order to evaluate the role of antigen variation and different strategies will have to be adopted in individual cases.

3. Complex pathogens (protozoa)

The more complex pathogens provide a particularly challenging problem since they play such an important medical role and since so little is known about their basic molecular biology. A problem is that they often are carried by vectors. The pathogen will then appear in different shapes during its life cycle in the human body and in the insect. The different stages of the parasite have different antigenic properties which hamper the development of

vaccines by genetic engineering. The genomes of the protozoa are moreover very complex which makes it difficult to identify the gene or the genes which are needed for proper antigen expression. A strategy for development of vaccines against complex pathogens would include the following components:

1. Facilities for growth of the pathogen in sufficient quantities for DNA, mRNA and antigen production.
2. The use of animal models in order to identify the protecting antigen(s).
3. The production of monoclonal antibodies for identification of protecting antibodies.
4. Basic studies on the structure and exresssion of genes in the pathogen.
5. Structural studies of parasite antigens, including protein sequencing.
6. mRNA fractionation and in vitro translation. Construction of gene and cDNA libraries.
7. Identification of the proper gene(s). This is a difficult task and several different strategies may have to be considered like selection of mRNA on cloned DNA followed by immunoprecipitation with monoclonal antibodies. Alternatively gene transfer could be used followed by identification of specific surface antigens. Synthetic oligonucleotides could also be used for the identification provided that part of the protein sequence is known.
8. Once the gene(s) are identified a study must be undertaken in order to achieve expression of antigen(s) that are suitable for vaccine production.

It should be emphasized that amazing progress has been made towards the construction of a vaccine against malaria, although this was considered to be an extremely difficult task. It is thus impossible to predict how fast the research frontier will move in the vaccine field.

A:4) Design of live vaccines

One problem which is connected with the production of human vaccines against prevalent tropical diseases is the requirement for vast amounts of vaccine to be distributed to hundreds of millions of people. A way to alleviate this problem would be to design live vaccines. In this way the administration of the vaccine would be

facilitated and a much larger fraction of the population could be treated without establishing a complicated distribution network. Also a better immune-status is likely to be achieved. Recently very promising results have been obtained concerning the use of live vaccines. The best known example is the use of the vaccinia virus, a non-pathogenic organism, which successfully has been used to eradiate smallpox. Vaccinia virus contains a very large genome into which foreign genes can be fused. An american team of scientists have introduced genes from hepatitis B virus into the vaccinia virus genome and in this way constructed a chimeric virus which is able to replicate in human cells and which during replication expresses the hepatitis B virus surface antigen. This leads to the induction of antibodies against both vaccinia virus and hepatitis virus antigens. Very promising results have also been obtained with similar chimeric viruses containing genes from Herpes Simplex virus. The use of a live vaccine has many advantages besides the practical ones. Since a "natural" immunogen is offered to the immune system a more potent and long lasting immune response is expected. The experiments which so far have been carried out utilizing the vaccinia virus genome as a vector obviously offers a lot of promise for the future.

Live vaccines could possibly also be constructed for protection against small viruses like enteroviruses which also are of paramount medical importance. A conceivable possibility is to replace the coat of a non-pathogenic virus with a coat from a pathogenic virus and in this way construct artificial viral strains which cause immunity without causing disease.

The design of live vaccines by genetic engineering is obviously a field of biomedical research which should be given high priority in the near future.

A:5) Construction of multivalent vaccines

Yet another advantage with genetic engineering techniques is that several genes can be combined in an artificial way. In this way it may be possible to produce a chimeric gene which encodes antigenic determinants representing several different viruses. By immunizing with such antigens it may thus be possible to induce protection against several unrelated microorganisms by one single injection.

A problem which hampers the potential application of products from genetically engineered bacteria is that the proteins sometimes induce a poor and short-lasting immunity. This is probably due to the fact that the artifically produced protein has a different conformation than the antigen which is present in the virus coat. Little is known about how the immune system recognizes conformational differences in proteins and much work on the immune mechanisms is clearly needed in the future before the genetic engineering can be applied in its full power to vaccine production.

A:6) Alternative procedures for vaccine production Recently an alternative procedure for the production of specific antibodies has been described. If the amino acid sequences is known for an antigen it is possible to synthesize a short peptide which is identical with a region in the antigen. Such peptides can be injected into animals for antibody production. Methods are currently available for chemical synthesis of short peptides. This procedure has been successfully applied to the production of specific antibodies against some rare antigens. Its potential use for vaccine production is currently under intensive study.

B) Production of peptides and proteins which are used as drugs in human and veterinary medicine

Many biologically active molecules are today extracted from cadavers in small quantities and in bad yields and their utilization is thus limited. In other cases animal tissues are used to extract the biological compound. In this case a major disadvantage is that a foreign protein will be introduced into a human host with complications from the immune system. In other cases when, for instance, blood products are used in therapy there is a considerable risk that infectious agents like Hepatitis B virus or more recently AIDS are transmitted to the patients. Genetic engineering offers possibilities to circumvent all these shortcomings. Essentially any protein compound can be produced by genetic engineering in virtually unlimited quantities and a stable supply of the pharmaceutical can thus be assured. The principle behind the use of genetic engineering for protein production was outlined in detail in section A, describing the use of genetic engineering for vaccine production. There are no essential differences, except

that in certain instances huge quantities of material will be needed which in turn will require carefully designed micro-organisms which secrete the protin into the growth medium. Important problems which are related to the use of genetic engineering for production of peptides and proteins is related to one or more of the following factors.

a) A need for efficient expression of genes in a foreign environment.

b) Degradation of foreign proteins in the host which is particularly relevant for proteins of large size.

c) Lack of modificaiton such as glycosylation, fosforylation or cleavage in procaryotic hosts.

d) Lack of suitable hosts which efficiently secret proteins into the growth medium.

e) Lack of organisms which are cheep to grow in large quantities.

f) Problems related to the stability of recombinant molecules containing large segments of foreign DNA.

The application of genetic engineering to the production of peptides and proteins for pharmaceutical use has progressed very rapidly. Bacterial strains which produce compounds like somatostatin, human growth hormone, human insulin and several kinds of interferon are already available. Methods for purification of the products from the bacteria have been developed so that virtually homogeneous protein or peptide preparations can be obtained. Also vectors which express the desired product in large quantities have been constructed. Clinical trials have been undertaken in several cases and the first drugs which are based on genetic engineering are just about to enter the market in several countries. There is thus no doubt that genetic engineering will play a paramount role in this sector of the pharmaceutical industry.

The problems which have been attacked so far have primarily concerned short peptides or small proteins. These are more easy to manufacture on a large scale in microorganisms due to their size.

The above mentioned examples, i.e. growth hormone, insulins etc. have also the advantage that their mRNAs are relatively abundant in certain tissues which makes it comparatively easy to isolate cDNA clones. In other cases it can be considerably more difficult to find the right gene to transfer to the microorganism

for expression. Many procedures have been developed to circumvent the problems. In some cases, completely synthetic genes are inserted in bacteria to be expressed. In other cases synthetic nucleotides are constructed on the basis of the protein sequence and then used for identification of the relevant DNA sequences. The research frontier is advancing very quickly in this gector of recombinant DNA technology. Several companies have already started to work on extremely complicated proteins like the blood coagulation factor VIII. In this case the problem is of a different magnitude since the protein is present in extremely small amounts in serum which makes it difficult to study the protein structure.

The use of genetic engineering for direct production of compounds is limited to proteins and peptides. Very small peptides are more easy to manufacture by chemical synthesis.

Genetic engineering can obviously not be used directly for production of other macromolecules than peptides and proteins. Carbohydrates are manufactured in the living cell by enzymes and are not coded directly in the DNA sequence. There is thus no possibility to transfer genetic information for specific polysaccharides directly to a microorganism. One way to circumvent the problem would be to allow microorganisms to produce the enzymes which are used in the biosynthetic pathway of the polysaccharide. There are no examples yet of successful production of macromolecules along this line. It is however likely that this kind of biosynthetic approach will play an important role in the pharmaceutical indystry in the future. It is discussed in more detail in section C.

C) Production of enzymes to be used for the biosynthesis and/or modification of drugs

Enzymes play an important role in the pharmaceutical industry today. They can be used in many different ways; one example is in replacement therapy. A more important role is the use of enzymes for the biosynthesis and modification of drugs. It is today a well known fact that many steroid hormones are produced by the use of enzymatic reactions. Also many antibiotics are produced by utilizing enzymatic pathways. Enzymatic procedures offer many advantages as compared to chemical reactions; i.e. they may be

cheaper and more efficient and do not require extreme physical conditions. It is to be expected that enzymatic modification of drugs will play an increased role in the future in the pharmaceutical industry. Genetic engineering will most likely make valuable enzymes available in large quantities and at a reduced price. Also enzymes which today are difficult or impossible to produce in sufficient quantities for practical use at an industrial scale will be available thanks to genetic engineering.

The proceedures for production of enzymes by gene technology do not significantly differ from those used for production of peptides, bulk proteins or vaccines. (see sections A and B). Generally the gene of interest is introduced into suitable microbial host and sufficient expression is obtained by the use of strong promoters. Bacteria as well as yeast are likely to be used as hosts for enzyme production whereas animal cells probably will turn out to be too complicated and too expensive to use. A clear advantage for the production of enzymes will be the use of microbes which secrete the enzyme into the growth medium. In many cases it will not be necessary to purify the activity and crude lysates of bacteria or preparations of growth medium can probably be used for biosynthetic purposes.

D) Protein engineering

Techniques are now available not only to isolate and characterize genes by molecular cloning but also to introduce specific alterations in the genetic contents of recombinant DNA molecules. Such alterations offer several advantagous effects which can be utilized by the pharmaceutical industry.

a) Point mutations can be introduced in the coding sequence to alter the structure of a protein. This technology will for instance make it possible to construct enzymes with new or altered specificities.

b) Termination codons can be introduced into a coding sequence so that truncated proteins are formed.

c) Two different genes can be fused to create chimeric proteins. In this way two or more functions can be combined in the same molecule.

Protein engineering also offers spectacular advantages for studies on the mechanisms of enzyme function. By replacement of specific amino acids detailed information can be collected about how enzymes carry out their important functions. In a long term perspective it should be possible to alter the specificity of enzymes by making amino acid replacements and possibly also to create enzymes with completely new specificities.

The technology also offers possibilities to specifically alter pharmaceutically active compounds other than enzymes. Their activity may be increased, their half life could be decreased or increased by alterations in the molecule and new biological activities could possibly be created which have not existed in nature before. This kind of engineering has just started but is likely to play an important role in biotechnology in the future.

E) Cell biology

This is a particularly important area of future research for the pharmaceutical industry. Thanks to gene technology eukaryotic genes and their protein products can be isolated, analyzed and expressed in large quantitites. Our current knowledge about the genetics and biochemistry of the animal cell is very limited. Genetic engineering provides novel and very powerful tools to study the structure and metabolism of animal cells. Once our knowledge about how cells function compounds are likely to be identified which can be used as pharmaceuticals. An example of a rapidly expanding field in this area are the growth factors. Genes for several growth factors have now been cloned and some of them already expressed in bacteria and yeast. This will provide a potential tool to influence biological processes like wound and bone healing etc.

The field of molecular immunology is another example of a rapidly expanding field where compounds are likely to be identified which will offer new therapeutic possibilities in connection with immunological disorders. By the use of growth factors and other triggers it may eventually become possible to master the different cellular constituents of the immune system.

The pharmaceutical industry should give high priority to basic research in cell biology since novel pharmaceuticals are very likely to be the result of such efforts. It must, however, be considered to be a long range goal.

F) Cell technology

Cell technology can be defined as the manipulation of whole cells. This technology offers several interesting prospects: one is changing the internal contents of the cell by inplanting new genes into the cells. The foreign genetic material can be injected into a cell nucleus through microinjection techniques using ultrafine needles. Techniques are also being developed to deliver the material inside the cell by enclosing it in microscopically small packages known as liposomes, made up of fatty substances that easily merge with cell-membranes. Another aspect of cell technology is the growing of multicellular tissues from a single cell. This has so far been accomplished with plants in which case the whole plant can be grown from a single cell. This is of course not possible with animals.

A striking example of successful cell technology is the fusing of two cells to make hybrid cells. Thus, if a cell making an important product cannot be grown it can be fused with another cell that is able to grow under laboratory conditions. A hybrid may in this way be obtained that makes the required product. This principle has lead to a breakthrough in the manufacturing of antibody. Antibodies are normally obtained from the blood of animals. However, by cell technology an immortal tumor cell with a potential for making antibodies can be fused with a cell that is actually making the required antibody. The hybrid cell then continues to multiply and gives rise to a clone of cells that grow easly like tumor cells but produce the required antibody. This technique of making so called hybridomas to produce antibodies is expected have far reaching consequences in medicine.

In general it is simple to fuse two cells by using agents such as viruses and chemicals that intermingle membranes of the two cells. However, only the hybrid made from cells of a single animal species may remain properly functional. The hybrids made from cells obtained from two different species are ususally not stable. However, since cells of two species can be fused it may be possible to derive functional hybrids in limited sence. Some properties of one cell may be transferred to another. This is particularly important in plants since whole plants can be grown from a single cell. Thus, cells of different plants may be fused to derive a plant with useful properties.

Cell technology offers several potential advantages to the pharmaceutical industry. One is the use of tissue culture systems to produce pharmaceutically active substances. For instance, by cultivating plant cells in a large scale many drugs which today have to be extracted from whole plants could be produced in larger quantities to a lower price. Moreover the use of animal cell cultures offers also several potential advantages. Genes can be introduced into animal cells by microinjection or transfection and in this way cell lines could be produced which continuously secret foreign substances of pharmaceutical value. This could also be extended to the introduction of foreign genes into whole animals.

G) Application of recombinant DNA technology to microbial genetics

Genetic engineering is an efficient tool to study the replication of viruses and microorganisms. By cloning relevant sequences from viral genomes it is possible to identify sequences of regulatory importance and also proteins which carry out regulatory and specific metabolic functions. In this way it should be possible to identify unique biosynthetic pathways in pathogenic microbes. This may allow the design of specific drugs which inhibit microbial replication without perturbing the metabolism of the host. This should in a longer perspective offer alternative possibilities to tailor specific drugs against viruses, protozoa as well as bacteria.

H) The use of recombinant DNA technology for genetic diagnostics and identification of microorganisms

Nucleic acid hybridization is a powerful tool to identify specific nucleic acids in crude samples. Microbial diagnostics have by tradition been based primarily on immunological proceedures. Recombinant DNA technology offers the possibility to isolate and manufacture specific DNA segments from microbial genomes. These can then be used in specific hybridization assays to detect the presence of a microorganism in clinical specimens. Nucleic acid hybridization procedures offer several advantages, one important being the fact that specific probes can be designed which are correlated to the pathogenicity of the microorganism rather than to the immunological properties of the surface of the microorganism.

Genetic disorders play an important role in the developing countries; sickle-cell anemia and the thalassameias being examples of such diseases. Recombinant DNA technology offers new possibilities to identify carriers of genes which are connected with genetic diseases. This type of diagnosis is likely to play an increasing important role for the developing countries in the future. Recently proceedures have been designed which also allow the detection of point mutations in the human genome. In this way carriers of the sickle-cell gene and other point mutations can be identified and the procedure is also applicable to antenatal diagnosis.