BIOTECHNOLOGY AND DEVELOPMENT REVIEW

JUNE-DECEMBER, 1995

Role of Biotechnology in Health Care in India: Present and Future

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Introduction

Medicines constitute the single, least-expensive but the most essential items in any health care system. Depending upon the countries, they constitute 7 per cent to 20 per cent of the health care costs. Of all the different systems of the medicines deployed in human therapy, the allopathic system is by far the most scientifically studied. The pharmaceutical formulations in allopathic system are (a) derived from plant and animal sources as extracts and concentrates, (b) produced by chemical synthesis, (c) manufactured by fermentation through the conversion of substrates by biological agents into desired products, and (d) substances derived from other natural sources. With the advent of biological science and with the greater understanding of genes, proteins, carbohydrates, fats and lipids coupled with the increased knowledge in infectious microbes and the physiology of bodily regulations, the scope of understanding and tackling of the changes caused in human body by infectious agents or by other defects has become profound. Biotechnology which encompases the techniques related to understanding, regulation and production of various gene products, the understanding of immunology and hybridoma technology hold enormous promise to offer therapeutic and prophylactic solutions to several afflictions causing human sufferings.

Biotechnology is technique based. It encompases the processes requiring the use of recombinant DNA methods, hybridoma technology, immunological techniques and cell culture methods using viral, bacterial, microbial, plant and other cell lines. It also includes separation, purification as well as assay methods of biological materials within its purview. Within the scope of above in the health care area, the

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production of vaccines; diagnostics; bioactive therapeutic proteins; blood products; antibiotics by fermentation; and gene therapy are discussed. The current scenario, the special features and the future prospects of some of these biotechnology products in India in the above areas are discussed hereafter.

Vaccines

Vaccines are the most potent and cost effective strategy of fighting infectious diseases. The World Health Organization (WHO) established the Expanded Programme of Immunization (EPI) in 1974 with the objective of preventing six childhood diseases namely polio, tetanus, diphtheria, pertussis (whopping cough), tuberculosis and measles. The Indian Expanded Programme of Immunization (EPI) introduced in 1978 was against the five above childhood diseases excluding measles and including typhoid; measles was added to the programme in 1985 and typhoid was dropped out as the available whole cell inactivated vaccine was not quite effective. WHO has now recommended the addition of Hepatitis B to the EPI. The Indian EPI has to cater to about 23 million new borns against major childhood diseases and an equal number of pregnant women annually against tetanus, which calls for making available very large quantities of vaccines. Fortunately abundant capacities have already been created in the country for the production of vaccines against tetanus (T), diphtheria (D), pertussis (P) and combinations thereof (e.g. DPT and DT), BCG and measles. Recently a human diploid cell derived MMR vaccine plant has gone into the production. Sheep brain derived as well as cell culture based (chick embryo derived) rabies vaccines, yellow fever vaccine, Japanese Encephalitis vaccine, inactivated cholera and typhoid vaccines are also being produced locally. The typhoid and cholera vaccines locally produced are of poor efficacy and are also reactogenic; consequently these have been discorded from the national programme. The Vi antigen based injectable typhoid (1) and Ty21a based attenuated oral typhoid vaccine (1) with improved protecting abilities have been introduced recently. In addition to active vaccines, equine anti sera against tetanus, diphtheria, gas gangrene, rabies and shake-vanom are also being produced locally. A manufacturing unit by the name M/s. Bharat Immuno Biological Co. Ltd. (BIBCOL) with an annual production capacity of 100 million doses of oral polio vaccine has been set up by the Central Government at Bulandsahar (Uttar Pradesh) and the unit had gone into production from the end of 1995. Genetically engineered as well as human plasma derived Hepatitis B vaccines are being marketed in the country through imports. Similarly some quantities of human diploid cell culture based rabies vaccine and the improved cell-cultured vaccines against measles, mumps and rubella (MMR) and

influenza are being imported and consumed. The current imports of these vaccine are low primarily due to their high unit costs. The current consumption of these vaccines may individually vary between 150,000 to 600,000 doses per annum.

The present turnover (1994) of vaccines in the country at manufacturers' level is estimated to be of the order of Rs. 1130 million for active vaccines and about Rs. 240 million for sensitized equine antisera. The vaccine market is growing at the rate of 8-10 per cent annually with the equine antisera market showing negative growth (2).

The following Table-1 indicates the requirements of some of the more important vaccines for 1994-95 and 1999-2000 A.D. as estimated by the author (2).

Table - 1
Requirement of Important Vaccines

Sl. No.	Particulars of vaccines	Estimated requirement 1994-95	(Million doses) 1999-2000
1.	DPT	105	114
2.	DT	50	57
3.	Tetanus toxoid	75	100
4.	BCG	41	43
5.	Polio	105	134
6.	Measles	25	30
7.	MMR	5	7.50
8.	Rabies: Sheep brain based	4	4.5
	: Cell Cultured	3	5
9.	Hepatitis-B: Plasma derived	0.10	0.20
	: Recombinant	1	45*
10.	Typhoid (attenuated oral)	10	50*
11.	H. Influenza type B	1	5
12.	Meningitis	0.5	2

*If included in the EPI Vaccination Programme

There would be opportunities for the setting up of basic production facilities for Cell cultured Rabies, recombinant hepatitis and other vaccines as the demand is increasing and the current production base either does not exist or are inadequate for most of these vaccines; besides sale of these products is primarily through the private sector which can pay for higher unit costs.

Most of the vaccines currently produced commercially and utilised are manufactured based on our emperical understanding of the immunogenicity of the antigens. Some viral vaccines are being produced in well defined cell lines, e.g. polio, rabies, measles, MMR etc. Only one vaccine against hepatitis-B has been produced by recombinant DNA technology in yeast where well defined plasmid constructs coding for the surface antigen of HBV have been made and used. Our knowledge of antigenic epitopes is gaining momentum and various synthetic peptides of 10 to 30 aminoacids are being either synthesized or produced by r-DNA technology and are being experimented upon using potent adjuvents. Various safe DNA viruses like vaccinia virus are being genetically engineered to introduce antigenic constructs against specific diseases and are being evaluated. Recently a vaccine against rabies, constructed in vaccinia virus has been allowed for field conditions (3). Many such vaccines against animal diseases are expected to be field trialed although the chances of use of such vaccines to protect human are remote due to ethical reasons as well as due to our inadequate understanding of the long term effect of the engineered viruses on the facultative and other aspects of human. Cocktail vaccine therapy through parenteral route is being experimented to minimize the number of patient visits to physicians. DPT cocktailed with hepatitis, influenza, typhoid and meningitis could be tried. However the success would depend upon the antigenic response to individual antigens. It is however doubtful if with the present day knowledge of antigens, their presentation and processing methods by the body, it would be possible to generate antibody protection level to the desired levels against all the antigens in one shot. Besides parenteral application of processed antigens, efforts are being made to enable transdermal applications through ultraflexible vehicles called transfersomes or liposomes to ellicit immunogenic responses and thereby to vaccinate individuals. This method is the extension of the work of transdermal presentation of insulin to diabetic patients (4). Efforts are also being made to deliver antigens through oral, nasal and occular routes. All these efforts are at the research stage. Our knowledge of understanding the method of presentation, processing and activation or inactivation of purified antigens by the body when presented through various routes, needs to expand and enlarge to enable real success in vaccination through these alternative new strategies.

The vaccines production status and the current global research strategy is briefly summarised below in Table - 2.

Table - 2

Vaccines: Production Status and Research Strategy

1. Conventional vaccines: (Most successful products, in commercial use)	Manufactured by experience and emperical knowledge e.g. small pox, cholera, typhoid, tetanus, diphtheria, pertussis, etc.
2. Purified viral vaccines in in-vitro cultured cell lines: (In extensive commercial use)	Production relies upon the knowledge of cell lines, culture methods and immunogenic agents e.g. measles, mumps, rubella, rabies, polio, etc.
3. Recombinant vaccines: (Most approaches in research stage)	Specific antigenic proteins and peptides expressed in engineered cell lines/microbes, e.g. HBV.
	Engineered viruses incorporated with genes coding for specific antigens, e.g. vaccinia incorporated rabies, F.M.D., etc.
4. Attenuation of pathogens in live microbes: (All in research stage)	Reassortment of gene segments, e.g. various live viral vaccines
	Genetic mutations, e.g. attenuated chorela vaccine, etc.

Diagnostics

The growth of diagnostics market is related to the prevalence and the incidence of diseases, the attitude of medical profession towards diagnosis, the per capita income as well as the paying capacity of individuals, Government policy towards national health programmes, the status of the Indian diagnostic industry (which supplies the products) as well as the extent of medical facilities available, specially in terms of pathological laboratories established throughout the country. India is burdened with a large number of communicable as well as non-communicable diseases. Besides, increase in the population growth is also creating enormous strain on the economy. In this context, simple, easy-to-use, inexpensive diagnostic kits for detection of diseases as well as the physiological status of the body fluid assumes great relevance and significance.

The turnover of diagnostics industry in India was about Rs. 1600 million during 1994. The market which was Rs. 200 million in 1987 had however, increased rapidly. The gradual increase in the budgetary provision in national health programmes is steadily helping the increased consumption of diagnostic devices and tests in public hospitals. There is also a sizeable portion of Indian population estimated to be about 180 million which utilizes 50 per cent of the national wealth itself. This sector of the population which is sizeable can pay for the expensive diagnostic tests whenever required. The scope for large consumption of diagnostics in this sizeable private market is also substantial. There are more than 11500 hospitals and more than 15000 pathological laboratories in the country which depicts the large infrastructure already available to support the local consumption of large volumes of diagnostics. It is, therefore, surmised that the diagnostics industry in the existing environment shall grow faster.

The current market is based on import intensiveness. The spectra of tests and kits marketed include clinical chemistry tests which are locally produced to a large extent. However, diagnostics which target the presence of specific antigens or antibodies by ELISA, EIA, RIA, Latex agglutination, etc. are mostly imported and marketed. Quantitative assay methods for hormones, cancer markers and specific enzymes are also imported and used. However, realizing the potential of this area the Government of India through its various agencies specially the Department of Biotechnology (DBT) are promoting research and developmental work for the indigenous development of techniques which could be used by the industry. These efforts are paying rich dividends and already techniques for the detection of seven conditions including early detection of pregnancy, detection of filariasis, amoebiasis, leishmaniasis, hepatitis B, typhoid fever and blood grouping methods have been developed in local institutions and transferred to the industry. Tests for a number of conditions are at various stages of development; it is anticipated that immunodiagnostic kits for about 15 conditions including methods for the early detection of tuberculosis, leprosy, toxoplasmosis, hepatitis, viral encephalitis, malaria, etc. would be locally developed and manufactured in India during the next 5 years.

The spectra of different classes of products marketed by the industry during 1994 in value as estimated by the author is given below (Table - 3).

Table - 3

Different Classes of Products Marketed : 1994

Sl No.	Classes of Products	Estimated Turnover 1994 (Rs. in million)	Turnover value as % of total
1.	Clinical chemistry Wet & dry reagents	448	28.0
2.	Dipsticks (blood & urine parameters)	128	8.0
3.	Immunodiagnostics for disease defection	461	28.5
4.	Hematology are blood grouping	269	17.0
5.	Microbiology & others	297	18.5
	Total	1600	100.0

Taking into consideration the rate of population growth in the country and the incidence of major diseases, the current potential number of tests for certain major conditions, would be as indicated below in Table - 4 (2).

Table - 4

Estimated Potential Number of Tests Currently Required

Particulars	Estimated current no. of tests required (potential) per annum (Nos. in million)
Early pregnancy	25-30
Tuberculosis	15-20
Leprosy	2-5
Typhoid Fever	3-5
Amoebiasis	20-25
Diarrhoeal Diseases (Rota virus, *E.coli*, etc)	5-10
Filariasis	20-25
Hepatitis B	10-12

Particulars	Estimated current no. of tests required (potential) per annum (Nos. in million)
HIV infection	10-12
Malaria	20-25
Veneral Diseases	10-12
Rheumatic Diseases	2-3
Cancer markers	2-3
Hormone Tests (hormones, T3, T4, TSH etc)	2.5-3
Ovulation Tests	2-3

It will be seen from the above that the current time is most conducive for the setting up of new production units in the country. As indicated earlier, the current rate of growth of the business is very high and there is a large private as well as Government market for the products. The infrastructure is also supportive. The Government is offering encouragement to the entrepreneurs by extending various supports including fiscal incentives. The R&D base in the country is also strong and there is a pool of trained man-power. Industrialists should take advantage of the current situation and develop basic manufacturing units in a phased manner.

Bioactive Therapeutic Proteins

Large scale production of therapeutic proteins which are required in substantial quantities, and which cannot be produced in abundant quantities from the traditional sources, has been facilitated by the use of recombinant DNA (r-DNA) technology. This technology has been used to clone genes either in simplified forms or genes have been engineered depending upon situations.

Therapeutic proteins have been isolated from non-human sources for use in human ailments as in bovine and/or porcine insulin for treating human diabetes. Use of non-human source proteins for therapy in human runs the risk of developing unwanted immunological responses. Consequently, the need for producing and using identical human proteins for human therapy assumes significance and in this direction the contributions of r-DNA technology would be invaluable.

The following Table-5 indicates the main r-DNA proteins which are already in therapeutic use (5,6).

Table - 5
Recombinant DNA Proteins Currently in Human Therapeutic Use

S. No.	Proteins	Use	r-DNA Sources
1.	Insulin*	Diabetes	*E.coli* & *Yeast*
2.	Human Growth Hormone	Dwarfism	Mouse Mammary Cells, *E. coli*
3.	Interferons*	Viral diseases, Cancers & AIDS	*E.coli*
4.	Interleukins	Various cancers	*E.coli*
5.	Tissue Plasminogen activators	Thrombolysis Ovary Cell line	Chinese Hamster Ovary Cell line
6.	Erythropoietin*	Anemia	Chinese Hamster Ovary Cell line
7.	Hepatitis-B* surface antigen	Vaccine against Hepatitis-B	Yeast
8.	Streptokinase	Thrombolysis	*E.coli*
9.	Epidermal Growth Factor	Wound healing including burns	*E.coli*
10.	Granulocyte Macro-* phage colony stimulating factor	Cancer, AIDS	Yeast
11.	Granulocyte * Colony stimulating factor	Cancer, AIDS bone marrow transplanation	Yeast
12.	Blood cloting * factor VIII	treatment of hemophelia	CHO cell line
13.	Bovine growth hormone	increase of milk yield	*E.coli*
14.	Tumor necrosis factor	sepsis, cancer	*E.coli*

Items approved for marketing in India.

A large number of human proteins are also in the making; several of these are in the advanced stages of development. Some of these having good market potential are mentioned below in Table-6. (5, 6).

Table - 6

Therapeutic Proteins in Advanced Stage of Development

S. No.	Proteins	Main Use	r-DNA Sources
1.	Atrial Natriuretic Factor (ANF)	Hypertension and Kidney diseases	Yeast
2.	Antithrombin III	Anticoagulant	CHO cell line
3.	Blood Factor VIII	Haemophilia	BHK-21 cell line
4.	Blood factor IX	Haemophilia-B	CHO cell line
5.	Epidermal Growth Factor (EGF)	Wound healing including burns	*E.coli*
6.	Human recombinant antibodies	Infections, Various Cancers	CHO cell line Mouse myeloma
7.	Hepatitis-B surface antigen	Vaccine against Hepatitis-B	C-127 cell line CHO cell line
8.	Plasminogen activator (including chimerics and modifications).	Thrombolysis	*E.coli* CHO cell line spodoptera frugiperda cell lines
9.	Superoxide dismutase	Cardiac treatment and Organ trans-plants	*E.coli*
10.	Tumor Necrosis Factor (TNF)	Antitumour and Antiviral Therapy	*E.coli*
11.	Urokinase	Thromobolysis	*E.coli*
12.	Fibroblast growth factor	neutopathic ulcers	*E.coli*/ Yeast

13.	Insulin like growth factor	osteoporosis, metabolism	*E.coli* Yeast
14.	Nerve growth factor	peripheral neuropathies	*E.coli* Yeast
15.	Platelet derived growth factor	diabetic ulcers	*E.coli* Yeast
16.	CD4 protein	AIDS vaccine	Mammalian cell line
17.	gp160 protein	AIDS vaccine	Mammalian cell line
18.	p24 protein	AIDS vaccine	Mammalian cell line
19.	Interleukin-1 receptor antagonist	severe sepsis	Mammalian cell line
20.	Atrial natriuretic	kidney failure	Mammalian cell line
21.	Ciliary neuro-tropic factor	amyotrophic sclerosis	Mammalian cell line
22.	DNase	cystic fibrosis	Mammalian cell line and yeast
23.	Leukocyte protease inhibitor	cystic fibrosis	Yeast
24.	Thymosin Alpha 1	Viral infection	*E.coli*

Recombinant proteins are usually made in micro-organisms like *E.coli* and yeasts. The advanced knowledge concerning genetics and physiology of *E.coli* has accounted for the preferential use of *E.coli* as a host for gene expression. Additional advantages include rapid generation of biomass due to high rates of cell growth and availability of low-cost culture conditions. Important drawbacks include its inability to exert certain post-translational modifications (e.g. disulfide bond formation, glycosylation and acetylation) which usually leads to wrong protein folding. In such situations, use of animal cell lines is preferred,

as otherwise the biological activity of the proteins gets substantially modified.

Recombinant DNA technology would be used as a substitute for the conventional methods, for production of bioactive molecules in unlimited quantities. Already several molecules like human insulin, interferon, human growth hormone, interleukins, epidermal growth factor, t-PA, urokinase, etc., have been produced on a commercial scale by r-DNA methods by incorporating the required genes into the various expression hosts as indicated above. The world market for r-DNA therapeutic proteins has been increasing at a galloping speed and the growth rate is estimated at more than 25 per cent per year. The most important products currently being marketed with high turnover are human insulin, hepatitis B vaccine, human growth hormone, interferons, tissue plasminogen activator and erythropoietin.

The race for producing bioactive molecules by r-DNA technology has started world over and this will soon be manifested by the production of many more numbers in the years to come. These would include broadly the neuroactive peptides, lymphokines and others regulatory proteins, cytokines, hormones, growth factors, colony stimulating factors and several vaccines against fatal diseases. Currently work is being pursued on over 300 different bioactive proteins world over.

The development in India in this emerging field is far behind the world standard as no product has yet been commercially made. The seven products indicated above are actually imported in finished forms and consumed. There are premier institutes such as the Institute of Microbial Technology, Chandigarh; Indian Institute of Science, Bangalore; National Institute of Immunology, New Delhi; Centre for Biochemical Technology, New Delhi; Vittal Malaya Scientific Research Foundation, Bangalore; and International Centre for Genetic Engineering and Biotechnology, New Delhi, where some initiative has been taken. Similarly, there are certain other universities and institutes like Delhi University, Delhi; Bose Institute, Calcutta; Jawaharlal Nehru University, New Delhi; Anna University, Madras; M.S. University, Baroda etc., where recombinant work has also been initiated. Prominent among these are the work for producing insulin, epidermal growth factor, streptokinase, bovine growth hormone, transforming growth factor, hCG, vaccinia virus incorporated rabies and hepatitis B antigens respectively. However, none of these work have reached the stage of commercial exploitation.

The work on downstream processing for isolating the bioactive molecules from recombinant micro-organisms on a large scale has not yet made any worth while progress. This area calls for developing

expertise for recovering milligram or microgram quantities (or even less) of materials from complex cell debri or cell soup; the techniques also require isolating the active materials from contaminated and unwanted biologicals. Considering the international developments in this field, it is high time that specialised R&D programmes are developed in the competent Indian institutes, either alone or in collaboration with the other developed countries to generate and strengthen the local capabilities. As these technologies are highly complex and equally highly paying, the companies possessing them are unlikely to part with these to the Indian industry. In the endeavour of searching for newer markets, the foreign companies may at best offer product marketing rights. Indeed, moves have already started in these directions and a few Indian companies have teamed up with foreign companies for marketing some of these products in India. Two companies have procured transformed *E.coli* and yeast hosts for producing interferons and hepatitis B surface antigen based proteins respectively from less known and unproven sources. The production technologies are being developed in India, using the transformed microbes.

While all these efforts would result in the development of a consumption market, the products would remain expensive if simultaneously indigenous capabilities of manufacture are not developed and local production facilities are not set up. It is foreseen that for marketing many of these life saving products, soon there would be investments for generating Phase-I, II and III clinical trial data based on materials produced from procured transformed hosts. Production facilities may be set up either in India or Indian partners may team up with small biotechnology companies for obtaining materials from abroad. Sophisticated quality control facilities as also some investment in the processing of crude proteins into finished products may take place during the next two to five years. The fate of these endeavour would shape the future investments in this very highly science based industry.

Blood Products

There is an acute shortage of blood in the country. As against the availability of nearly 2.5 million units annually, the requirement is at least about 4 million units. If the whole blood could be fractionated, it could serve the requirements of 2 to 3 times the present beneficiaries.

The main fractions derived from the blood that are extensively used by the medical profession are serum albumin, gamma globulins and antihemophilic factors. The production of various blood products calls for superior technological innovations specially on separation techniques. Of the many methods, the modern chromatographic techniques have

played the leading role in the separation and purification of various products from the plasma. Any method in use has to satisfy the stability of the finished products; the yield must be reasonable to justify the processing costs, the processing time and its practically; ultimately the cost of processing must be carefully examined for deciding about its commercial applicability. Chromatographic methods have been found to be satisfactory when judged from the above mentioned criteria. The schemes generally applied to the separation of blood products from plasma are summarized in Figure - I (7).

A processing plant for the production of albumin, IgG and Factor-IX has been set up recently at K.E.M. Medical College, Bombay. The unit can process nearly 10,000 litres of blood annually. However, due to non-availability of adequate blood, the facility runs at lower capacity. There is a need to install such facilities in 4 to 5 regions in the country to enable better (about 300 ml is one unit) of blood are sold at Rs. 350 to Rs. 700. If blood could be separated into its cellular and serum components, one unit could be utilised for 4 to 5 patients. There is a need to have political will and societal commitment to enable the procurement of disease free blood from health donors in the country to enable the country to contain the acute shortage of blood felt in most of the hospitals in India. Production units could be set up to run profitably if proper policy measures are framed and if such units are run by the Government.

Antibiotics

In the area of antibiotic production, India has made considerable progress. Currently, the country consumes about 30 bulk antibiotics and produces 14 numbers by fermentation. The major basic manufacturing plants set up are for the production of Penicillins, Streptomycin, Erythromycin, Tetracycline, Ampicillin, Amoxycillin, Cephalexin and Gentamicin. Large quantities of Chloramphenicol are being produced from the imported intermediates. Two plants for the basic production of Rifampicin by fermentation have been set up which became operational from the middle of 1993 and one new unit is under construction. Three new Pencillin G first crystal units have been under operation from 1995 and three existing ones are under substantial expansion.

The consumption as well as demand of antibiotics in the country is increasing fast as it would be seen from the following Table-7 (2).

Table - 7

Projected Requirement of Antibiotics

Product Category		Requirement during 1994-95	1999-2000
Penicillin G/V	MMU	3040	6300
Injectable Cephalosporins	MT	25	60
Tetracycline	MT	310	300
Oxytetracycline	MT	200	200
Gentamicin	Kg	8860	4900
Erythromycin	MT	100	150
Rifampicin	MT	220	300

It is anticipated that the betalactams would continue to maintain their lion share in the market in the coming years over the macrolides, the tetracyclines and the aminoglycosides. For reasons of wide spread prevalence of tuberculosis, the use of rifampicin (along with other synthetic drugs) shall continue to increase at least during the next one decade till the health care infrastructure improves, and till the alternative more effective drugs emerge. Among the betalactams, the consumption of oral cephalosporins would increase significantly. The cephalosporin C as well as the Cephamycin C derived injectable Cephalosporins would also increase sizeably. However, the 6-APA as well as the 7-ADCA derived betalactums, both synthesised from Penicillin G/V first crystals would continue to dominate the scene as these are more cost effective cures with reasonably high degrees of success against a wide spectra of bacterial diseases.

Gene Therapy

The technique involves correcting gene defects in somatic cells or in germ lines. Currently more than 3500 genetic diseases are known which involve defects in at least one gene. The major single-gene-defect-related diseases are thalassaemia, sickle cell anaemia, inherited blood clotting disorders, severe combined immunodeficiency disorders

(SCID) hypercholesterolaemia and cystic fibrosis. The present day knowledge enables us to splice and isolate specific human genes alongwith the neighbouring regions of DNA which control their expression. Therefore, there are good possibilities of replacing defective genes alongwith the promoter sequences and replace them by the corrected genes and DNA sequences. For correcting single gene defects in somatic cells, the latter are manipulated *in-vitro* by using engineered retro viruses and then the transformed cells are replaced into the body and allowed to colonise. Other methods of gene transfer are by liposomes, killed Sendai virus envelopes packed with DNA, etc. Currently besides *in-vitro* methods, several *in-vivo* methods are also in practice which are gaining more acceptance. Various methods (8) for *in-vivo* gene delivery are illustrated in Fig-2 schematically by using the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) as the example.

Besides somatic cells, correct genes/sequences could also be incorporated into the embryos after *in-vitro* fertilization, or after *in-vivo* fertilization, flushing out the embryos and incorporating the target genes *in-vitro* followed by implantation of the transformed embryos into the uterus.

Polygenic disorders are complex and the mechanism of regulations are not well understood. Polygenic disorders are attributed to diabetes, rheumatoid disorders, proneness to heart attacks, psychitric diseases, alcoholism, and all kinds of cancer. Such disorders may be inherited or may be triggered by environmental stresses. Very little success has yet been achieved in correcting polygenic disorders.

Gene therapy is in the experimental stage world over. The experiments on human encompasses several ethical issues such as the scope of prenatal diagnosis and screening, commercial exploitation of new findings, commercial use of human genes/genome and eugenic issues. Indeed the science is so new and complex that development in most of the countries including all the developing countries is inadequate. Consequently most of the developing countries have not only started gene therapy experiments in human, they have not yet even framed their ethical guidelines for conducting such experiments in human subjects. In India, for example, there is not yet any officially approved guidelines although some human trial experiments at the Cancer Research Institute, Bombay on polygenic tobacco linked oral cancer are expected to be started shortly (9). The Indian strategy is to introduce HStk gene into the tumor cells through retrovirus mediated transfer, and make them susceptible to GANCICLOVIR, an antiviral drug. The experiments are still at the trial stages, and are being perfected in nude mice.

It is anticipated that with our more understanding of splicing, cloning, expression and regulation of human genes in cells, the gene therapy for single gene defects in somatic cells would become a reality in the developed world. The Table - 8 lists the potential candidates of the earliest gene therapies for diseases caused by single gene defects and their status of development(10-14).

Table - 8

Potential Candidates for the Earliest Gene Therapies

(For disorders caused by defects in a single gene that has been cloned)

Disorder	Incidence	Normal Product of defective gene	Target Cells	Status
Hemoglobino-pathies (Thelesse-mias)	1 in 600 in certain ethnic groups	Constituents of hemoglobin	Bone marrow cells	needs to be improved
Severe combined immunode-ficiency (SCID)	rare	adenosine deaminase (ADA)	Bone marrow cells of T lymphocytes	clinical trial underway in USA
Hemophilia B	1 in 30000 males	Blood clotting factor IX	liver cells of fibro-blasts	good chances for trials
Familial hypercholes-terolemia	1 in 500	Liver receptor for LDL	liver cells	animal studies in progress
Cystic fibrosis	1 in 2500 caucasians	substance imp. for keeping the airway epithe-lial cells free of mucus	lung cells	clinical trials in USA

Duchenne's muscular dystrophy	1 in 10000 males	Dystrophin (structural component of muscles)	muscle cells	work in progress
Diabetes type I	1 in 500	insulin	liver cells	animal studies in progress

Diagnostic devices based on genetic probes would also be available for diagnosing a large number of genetic disorders. It would take some time however to extend the technique to correct the germ line defects, as the ethical issues would be required to be resolved first. Gene therapy in polygenic disorders would take several years to be perfected as our understanding of the complex regulations and inter-relationships of multigenes is yet at a rudimentary stage.

Conclusions

The use of biotechnology in Indian health care industry is significant in the areas of vaccines, diagnostics and antibiotics. Currently certain bioactive therapeutic proteins are also being consumed locally after importing the finished formulations; the consumption of these products is however yet low, but would increase fast if the prices come down significantly from the current levels. These would be possible as the technology becomes wide spread, many production units are set up and when there is steep competition in the market place.

The demand of most of the biotechnology products is increasing significantly. There is also no price control on many of these products and in some there is significant price protection. Consequently, substantial investment is contemplated in several areas. New investments are anticipated for the setting up of production units for the diagnostics, vaccines and antibiotics. There would also be substantial expansion of certain existing units. A few processing plants for the separation of blood into cellular components as well as the processing of plasma for the production of albumin, immunoglobulins and blood factors could be set up if appropriate policies are made to encourage donation of blood from health volunteers; this would ease the acute shortage of blood in hospitals. Technologies for certain recombinant proteins may come to India and the first production plant may be operational in 1997.

The success of induction of a modern biotechnology industrial base into the present Indian pharmaceutical sector would stem from the sector's constant preparedness to look for newer opportunities and its abilities to capture, assimilate and adopt these opportunities as rapidly as possible. The capability would largely depend upon its own strength of R&D which is currently weak and needs substantial strengthening in terms of investment as well as capability building. In addition to strengthening the industrial R&D base, the capacities are to be strengthened in the existing functional capabilities in marketing, production infrastructure and information generation on safety aspects of products. Smaller marketing companies would deal with small volume products only like diagnostics, speciality instruments and devices as well as special plastics. However, the marketing of every biotechnology product of *in vivo* use would need the scientific inputs of highest capabilities. The products would often be sensitive to temperature fluctuations and would thus require an efficient cold chain for stocking and distribution which would again be expensive. Establishing a sizeable market demand may follow the setting up of basic production facilities in India. Most of the modern biotechnology products currently available are applied by injection. Creation of such facilities would again require sizeable investments and only the companies with command on large resources and high capabilities in biotechnology would be able to take up such ventures. In the meantime, the consumption market for the biotech products in all the sectors would keep on increasing steadily in India (15) as in the world.

Acknowledgements:

Thanks are due Dr. T.V. Ramanaiah, Senior Scientific Officer in the Department of Biotechnology for his kindly reading the manuscript carefully and providing helpful suggestions.

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FIGURE - 1

SCHEMATIC FLOWSHEET FOR FRACTIONATION OF BLOOD PLASMA

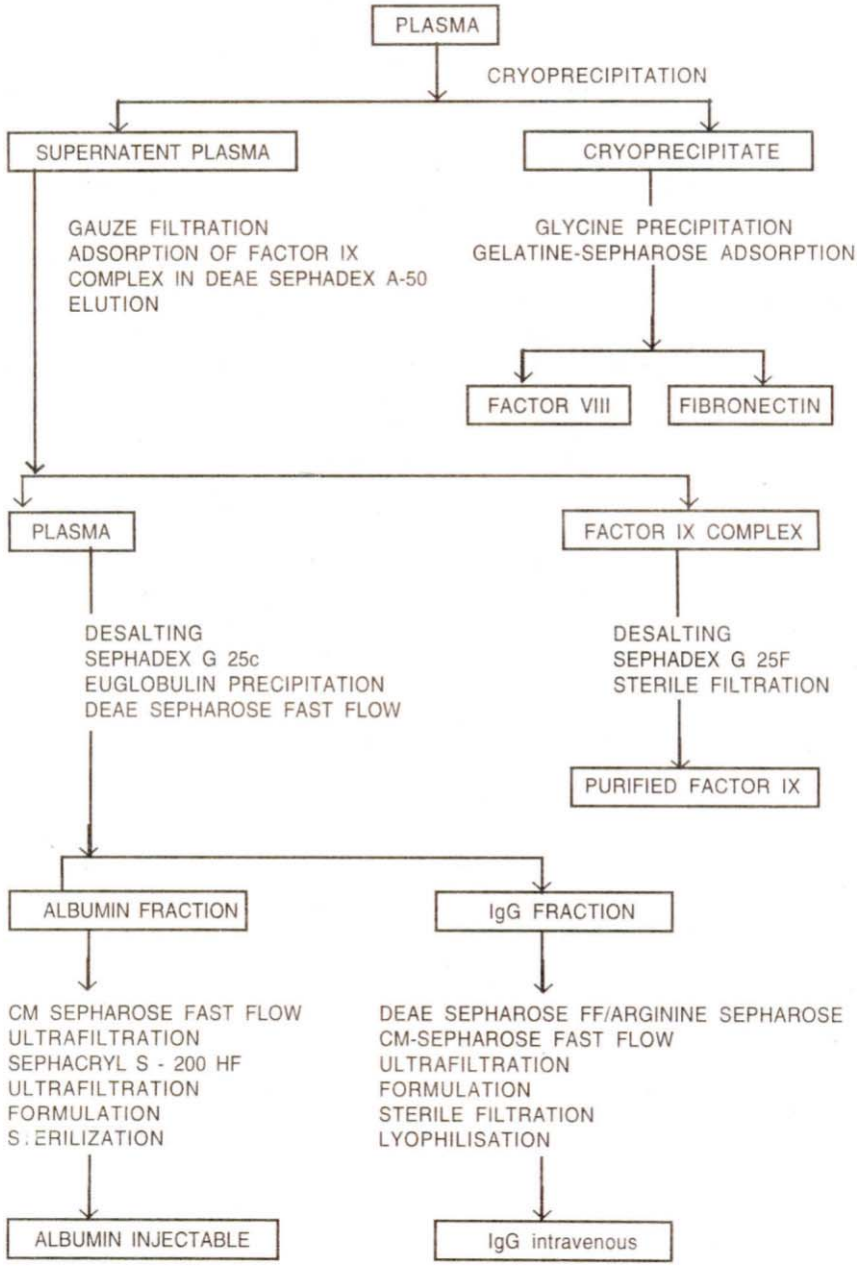
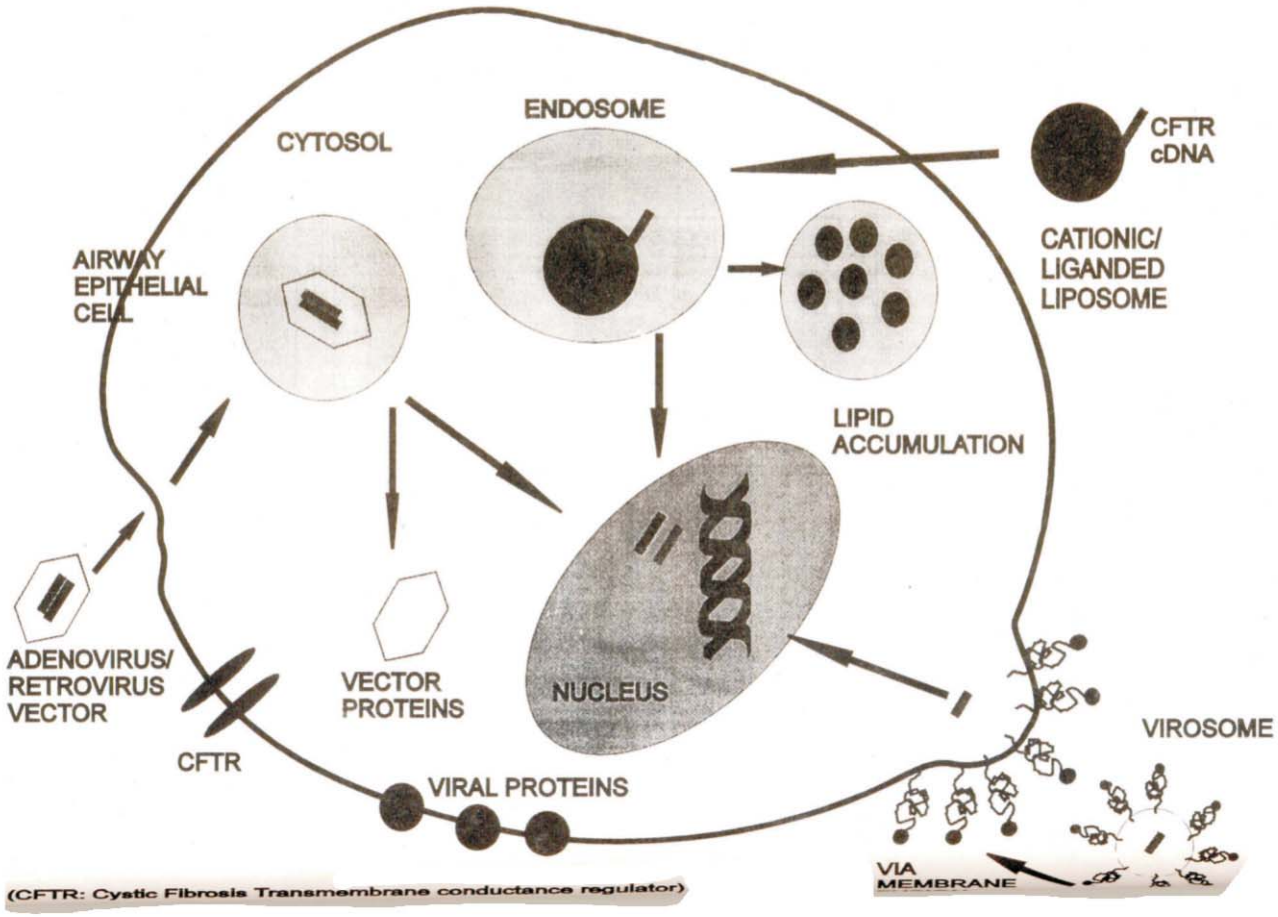


FIGURE 2: METHODS FOR GENE DELIVERY USING CFTR GENE AS EXAMPLE



(CFTR: Cystic Fibrosis Transmembrane conductance regulator)