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Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

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At least 1500 distinguishable human diseases are already known to be genetically determined (*1*), and new examples are being reported every year. Many human genetic diseases are rare. For example, the incidence of phenylketonuria is about one per 18,000 live births or about 200 to 300 cases per year in the United States (*2*). Others, such as cystic fibrosis of the pancreas, occur about once in every 2500 live births (*3*). When considered together as a group, however, genetic diseases of humans are becoming an increasingly visible and significant medical problem, at least in the developed countries. While the molecular basis for most of these diseases is not yet understood, a recent review (*4*) listed 92 human disorders for which a genetically determined specific enzyme deficiency has been identified.

Concurrent with the recent progress toward biochemical characterization of human genetic diseases have been the dramatic advances in our understanding of the structure and function of the genetic material, DNA, and our ability to manipulate it in the test tube. Within the last 3 years, both the isolation of a piece of DNA containing a specific group of bacterial genes (*5*) and the complete chemical synthesis of the gene for yeast alanine transfer RNA (*6*) have been reported. These advances have led to proposals (*7*) that exogenous "good" DNA be used to replace the defective DNA in those who suffer from genetic defects. In fact, a first attempt to treat patients suffering from a human genetic disease with foreign DNA has already been made (*8*).

Nevertheless, we believe that examination of the current possibilities for DNA-mediated genetic change in humans in the light of some of the requirements for an ethically acceptable medical treatment raises difficult questions. In order to focus the discussion, in this article we concentrate on the prospects for using isolated DNA segments or mammalian viruses as vectors in gene therapy. For this reason we do not discuss other techniques, such as cell hybridization (*9*), which have been used to introduce new genetic material into mammalian cells. We limit our discussion to the possible therapeutic uses of genetic engineering in humans. The potential eugenic uses, for example, the improvement of human intelligence or other traits, are not discussed because they will be very much more difficult to accomplish (*10*) and raise rather different ethical questions. Whether genetic engineering techniques can be developed for therapeutic purposes in human patients without leading to eugenic uses is an important question, but lies mostly beyond the scope of this article.

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Schematic Model of Genetic Disease

Some aspects of a hypothetical human genetic disease in which an enzyme is defective are shown in Fig. 1. The consequences of a gene mutation which renders enzyme E_3 defective could be (i) failure to synthesize required compounds D and F; (ii) accumulation of abnormally high concentrations of compound C and its further metabolites by other biochemical pathways; (iii) failure to regulate properly the activity of enzyme E_1, because of loss of the normal feedback inhibitor, compound F; and (iv) failure of a regulatory step in a linked pathway because of absence of compounds D or F, as in the increased synthesis of ketosteroids in the adrenogenital syndrome (*11*). In some cases of human genetic disease, accumulation of high concentrations of compound C and its metabolites appears to do the damage. Often a consequence is mental retardation.

The pathway in Fig. 1 is typical of some recessively inherited genetic defects which result in a deficiency of some gene product, usually an enzyme or hormone. In theory, such defects might be corrected by gene therapy, since such techniques might be able to restore the deficient gene product. Other kinds of genetic defects, including those such as the Marfan syndrome which show dominant inheritance and those such as Mongolism that are caused by chromosome abnormalities, could probably not be ameliorated by the kind of gene therapy we emphasize here.

Current Therapy

Human genetic diseases are usually treated by dietary therapy (*12*), drug therapy, or gene product replacement therapy (*11*). For example, diets low in lactose or phenylalanine are used as treatments for individuals with galactosemia and phenylketonuria, respectively. Such diets have proved exceedingly effective in galactosemia and have produced a marked reduction in the incidence of mental retardation associated with phenylketonuria. In terms of Fig. 1

this therapy corresponds to restricting the intake of compound A, thus minimizing the accumulation of compound C whose further normal metabolism is blocked.

Drug therapy has been used to block or reduce the accumulation of undesired and possibly harmful metabolites. One example is the inhibition of the enzyme xanthine oxidase with the drug allopurinol to reduce the accumulation of uric acid associated with gout and the Lesch-Nyhan syndrome (*13*). At present, this method of treatment has been applied to only a few human genetic diseases. Its more general application clearly depends upon the availability of drugs which act selectively on specific enzymes. In another form of drug therapy, drugs which combine specifically with the accumulated compound C are used. An example is the use of D-penicillamine to promote excretion of excess cystine in patients with cystinuria (*11*).

In theory, some human genetic diseases might be alleviated by supplying directly the deficient enzyme (E_3 in Fig. 1). Recently, attempts to treat Fabry's disease (*14*), metachromatic leukodystrophy (*15*), and type 2 glycogenosis (*16*), by administering the missing enzyme have been reported. Since exogenous enzyme molecules are eventually inactivated or excreted from the body, repeated enzyme injections would be required to manage the diseases in this way. In time, the patients would probably respond by forming antibodies against the administered enzyme. However, insoluble or encapsulated enzyme preparations may in the future provide a means of supplying therapeutic enzymes in a more stable and perhaps less immunogenic form.

There are growing possibilities for the early detection of some genetic diseases by diagnosis in utero. It is now possible to sample the cells of a growing fetus in utero and by examining these cells to diagnose a variety of genetic defects (*17*). If genetic defects are detected, some states will permit an abortion if the prospective parents so desire. We recognize that diagnosis in utero and abortion raise difficult social and ethical problems of their own and cite them only to indicate that there are additional alternatives to prospective gene therapy for coping with human genetic disease.

However, many genetic diseases do not yet respond to any of these treatments. For example, most genetic disorders of amino acid metabolism (other than phenylketonuria) cannot be well controlled by dietary therapy. Storage diseases associated with lysosomal enzyme deficiencies (*18*) do not appear to respond to enzyme therapy (*14, 15*) and will probably be impossible to control by dietary restriction. In addition, even in cases where disease management is effective, it is seldom perfect. Individuals with diabetes mellitus, when treated with insulin, have an increased incidence of vascular and other disorders and a decreased life expectancy compared to the nondiabetic population (*19*). Children with Lesch-Nyhan syndrome may have their uric acid accumulation controlled by drug (allopurinol) therapy, but their brain dysfunction has, to date, not been reversible.

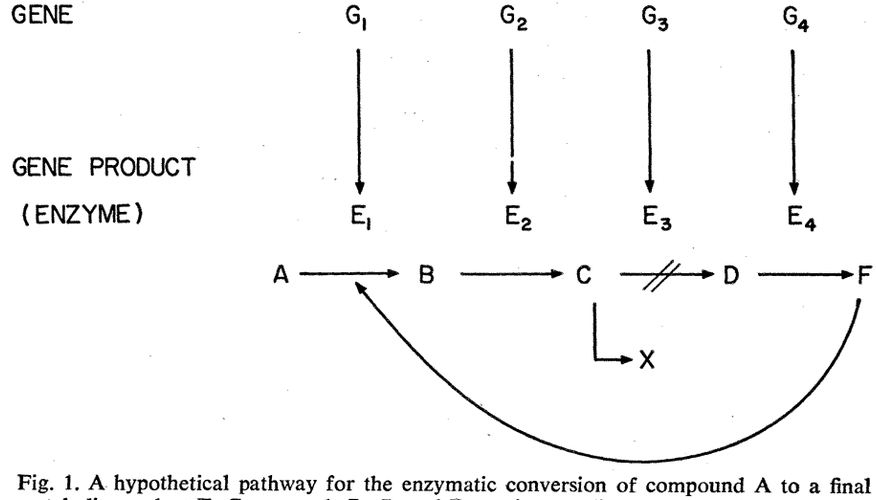

Fig. 1. A hypothetical pathway for the enzymatic conversion of compound A to a final metabolic product F. Compounds B, C, and D are intermediate products. Four different enzymes, E_1, E_2, E_3, and E_4, the products of the corresponding genes G_1, G_2, G_3, and G_4 are required to effect the conversion. The block occurs in the conversion of compound C to D. The concentration of compound F regulates the activity of the first enzyme in the pathway, E_1, in a feedback control loop.

These limitations of current therapy are stimulating attempts to develop techniques for treating human genetic diseases at the genetic level, the site of the primary defect. Genetic modification of specific characteristics of human cells by means of exogenous DNA seems possible for several reasons. DNA-mediated genetic modification of several different kinds of bacteria has been known for many years (*20*), and recent experiments suggest that the genetic properties of mutant *Drosophila* strains can be modified by treating their eggs with DNA extracted from other *Drosophila* strains (*21*). It has also been found that treatment of human cells in vitro with DNA extracted from the oncogenic virus SV40 results in permanent hereditable alteration of several cellular properties (*22*).

Genetic Modification Mediated by DNA

Permanent, heritable, genetic modification of a human cell by means of DNA requires (i) uptake of the exogenous DNA from the extracellular environment; (ii) survival of at least a portion of the DNA during its intracellular passage to the nucleus; (iii) stabilization of the exogenous DNA in the recipient cell; and (iv) expression of the new genes via transcription into an RNA message (mRNA) and translation of this message into the appropriate protein. Some of these processes are illustrated schematically in Fig. 2.

Mammalian cells take up proteins, nucleic acids, and viruses from their environment by a process known as endocytosis (*23*). After binding to the cell membrane, the macromolecules are drawn into the cell by an infolding of the external cell membrane leading to vesicle formation (see Fig. 2). Macromolecules contained in vesicles derived from invaginations of the external cell membrane can be degraded if these vesicles fuse with lysosomes. Lysosomes are cell organelles which contain a variety of hydrolytic enzymes. These enzymes can rapidly degrade ingested macromolecules, including DNA (*24*). Thus, mammalian cells possess mechanisms for protecting themselves from the potentially perturbing influences of foreign DNA.

Despite this cellular defense mechanism, exogenous foreign DNA can, under certain circumstances, become integrated in the DNA of the recipient cell. The evidence of this has come from stud-

ies of oncogenic virus transformation of mammalian, including human, cells (*25*). In the case of oncogenic transformation with SV40 virus, the viral DNA is apparently physically integrated into the chromosomal DNA of the recipient cell (*26*). It seems probable that heritable alterations of cell morphology and biochemistry are the result of the expression of one or more viral genes. Presumably, viral DNA integration takes place by base pairing of homologous regions of host cell and viral DNA followed by genetic recombination. However, the integration of oncogenic viral DNA may represent a special case since at least one viral gene product may be required for integration (*27*). Other, nonviral DNA molecules, unable to supply this integration function, might integrate at a much lower frequency, if at all.

In addition to integration by genetic recombination, exogenous DNA might be stabilized in the recipient mammalian cell as an independently replicating genetic unit in the cell nucleus. Although such units are known to exist in bacteria, they have not been observed in mammalian cells. However, the cytoplasmic mitochondria of mammalian cells do contain nonchromosomal, independently replicating units of DNA. The mitochondrial DNA replication system thus offers another possible site for stabilization of exogenous DNA.

For a human genetic defect to be repaired by administering exogenous DNA, the stabilized newly introduced DNA must be correctly expressed. That is, the new gene must be correctly transcribed into mRNA and this mRNA must be correctly translated into protein. Since little is known about the regulation of mRNA synthesis and translation during natural gene expression in mammalian cells, a corresponding high degree of uncertainty exists concerning the ability of newly introduced DNA to be expressed correctly.

A variety of attempts have been made to demonstrate DNA-mediated modification of genetically mutant mammalian cells, both in vivo and in vitro. Apparently successful results in vitro have been reported for diploid human cells lacking the purine "salvage" enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT); in human reticulocytes synthesizing an abnormal hemoglobin; in several malignant cells of mouse origin carrying markers for drug resistance; and in mouse cells with defective melanin synthesis, among others (*28*). In addition, transient expression of HGPRT enzyme function has been detected in human cells deficient in HGPRT after exposing them to DNA from cells with normal amounts of HGPRT (*29*). This suggests that exogenous DNA may be taken up and expressed, without necessarily being stabilized. However, none of the successful experiments described to date have been reproducible.

There may be several reasons for failure to demonstrate consistently the genetic modification of mammalian cells by DNA. Many previous experiments suffered from the unavailability of good genetic markers and sensitive selective systems for detecting modified cells. An important difficulty in using bulk DNA isolated from human (or other mammalian) cells is that the fraction of this DNA which is specific for any given gene is estimated to be extremely small, of the order of 10^{-7} (*30*). As we mentioned earlier, nonviral exogenous DNA may not be able to integrate into the chromosomal DNA of the recipient cell, thus preventing permanent genetic modification.

In spite of the lack of reproducible success in past experiments, several recent technical developments have suggested new ways in which the problems of low DNA specificity, failure of integration, and intracellular DNA degradation might be overcome.

The prospects for directing genetic modification of mammalian cells would almost certainly be enhanced by using DNA preparations containing only the gene for which the genetically defective cells are mutant. As already pointed out, both the isolation of a specific group of bacterial genes and the complete chemical synthesis of a single gene were reported recently (*5, 6*).

The RNA-dependent DNA polymerase recently found in RNA tumor viruses (*31*) could also be used for gene synthesis in vitro. Since this enzyme is able to make DNA copies from an RNA template, it offers a method for synthesizing the DNA for any specific RNA which might be isolated in pure form. Thus, it seems probable that our developing ability to isolate specific genes, or synthesize them, will eventually eliminate the problem of low specificity of the exogenous DNA.

Some workers are developing techniques which could be used to overcome the problem of stabilizing the incoming exogenous DNA in the recipient cell (*32*). They plan to make use of the ability of the DNA from SV40 virus to integrate into the chromosomal DNA of the cell. Specific genes will be attached to the viral DNA by means of several biochemical steps which are already known and fairly well characterized. These operations would create a hybrid DNA molecule which would carry the information for integration from the original viral DNA and perform the specific gene functions of the attached DNA. In this approach, DNA integration would be combined with biochemical manipulation of the DNA gene substance in vitro, and any gene-specific DNA segments obtained by synthesis or

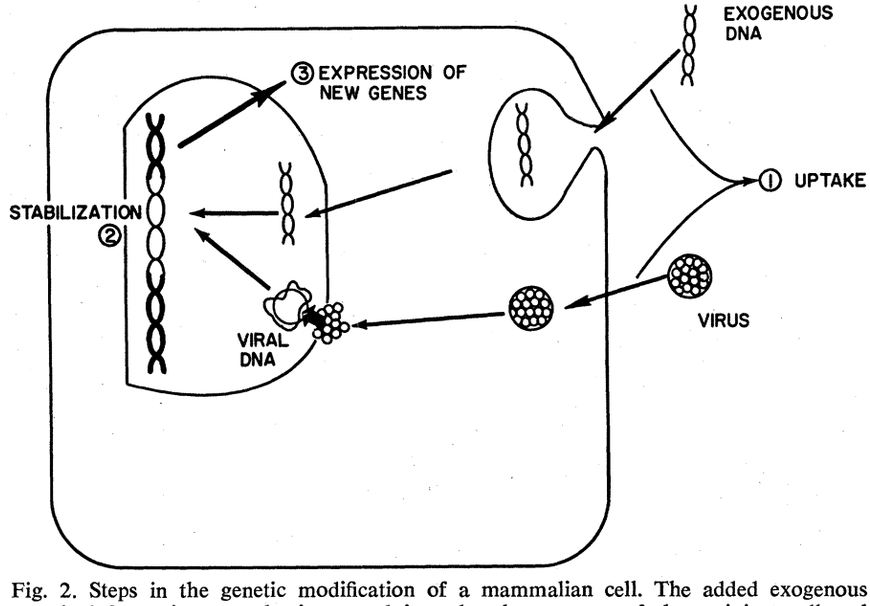

Fig. 2. Steps in the genetic modification of a mammalian cell. The added exogenous genetic information may be integrated into the chromosome of the recipient cell and become expressed as a new gene product.

isolation could be utilized. It is clear that before such hybrid DNA molecules could be used in a human therapeutic situation, the oncogenic potential of the viral DNA would have to be eliminated.

In another experimental approach, virus-like particles which contain pieces of cellular DNA (pseudovirions) instead of viral DNA are being used as the vector for DNA-mediated genetic modification (*33*). This might help to protect the incoming DNA from intracellular degradation. However, pseudovirion DNA is probably a random collection of cellular DNA fragments (*34*) and hence nonspecific for any given gene; it might also be unable to stabilize itself by integration into the host cell DNA. This may explain why attempts to modify thymidine kinase–deficient mouse cells in vitro by means of polyoma virus pseudovirions have been unsuccessful (*35*).

It has recently proved possible to reconstruct infectious particles of several plant and bacterial viruses from the nucleic acid and capsid protein components (*36*). This suggests the possibility of creating artificial pseudoviruses as vectors for DNA-mediated genetic modification. These pseudovirions would contain specific DNA segments (either isolated or synthesized) surrounded by virus capsid protein. The probability of introducing a specific piece of genetic material might be greatly increased when compared with natural pseudovirions carrying randomly excised pieces of DNA. This in no way solves the difficulties of integration and expression of the genetic material. Since the specificity of virus-cell interactions is determined at least in part by the virus capsid protein, encapsidation of specific DNA molecules might confer some cell or tissue selectivity upon the DNA molecules used for gene modification.

DNA-Mediated Gene Therapy

In attempting to envision how DNA might be used as a mediator for the modification of genes in a human being suffering from a genetic defect, we foresee several kinds of new problems. First, the existence of differentiation and cell specialization in the human body will pose several questions. Many human genes are active or expressed only in a small fraction of the cells of the body. For example, the activity of the enzyme phenylalanine hydroxylase (deficient in individuals with phenylketonuria) is demonstrable only in the liver. For prospective gene therapy there might be several consequences. (i) The introduction of, for example, the gene for phenylalanine hydroxylase into cells which do not normally express this enzyme would yield no therapeutic benefits if the expression of the newly introduced genes were also blocked. Methods would have to be developed to deliver the exogenous DNA to the appropriate "target tissue," and to confine its action solely to that tissue. (ii) Some gene products (hormones, for example) are made and secreted by one specialized group of cells and act on target cells elsewhere in the body. Synthesis and secretion of hormones such as insulin are regulated by mechanisms which are still imperfectly understood. Thus, the introduction of new genes for insulin into cells not appropriately differentiated to provide the correct synthetic and secretory responses would be of little use as a treatment for diabetes. (iii) In several genetic disorders, genetic modification of the brain cells themselves may be required to reduce the accumulation of metabolites in the brain, because the blood-brain barrier might prevent enzymes made in other parts of the body from entering the brain (*15*). We wonder whether direct genetic modification of brain cells could be made safe enough for use in human patients.

Second, regulation of the quantitative aspects of enzyme production may present a problem. By mechanisms as yet unknown, concentrations of cellular enzymes are regulated so that neither too much nor too little enzyme is produced by normal cells. How will we ensure that the correct amount of enzyme will be made from the newly introduced genes? Will the integration event, linking exogenous DNA to the DNA of the recipient cell, itself disturb other cellular regulatory circuits?

Third, the patient's immunological system must not recognize as foreign the enzyme produced under the direction of the newly introduced genes. If this occurred, the patient would form antibodies against the enzyme protein, perhaps nullifying the intended effects of the genetic intervention. This suggests that the new gene introduced during gene therapy would have to code for an enzyme with the same amino acid sequence as the human enzyme.

In addition, administration of foreign genetic material to patients carries a risk of altering the germ cells as well as the desired target cells. One might think that this problem could be circumvented by first removing some of the patients' cells, carrying out DNA-mediated genetic modification in vitro, and then reimplanting the altered cells back into the patient. However, this approach is likely to be limited by the tendency of cells to dedifferentiate and become malignant when grown in vitro.

For an acceptable genetic treatment of a human genetic defect, we would require that the gene therapy replace the functions of the defective gene segment without causing deleterious side effects either in the treated individual or in his future offspring. Years of work with tissue cultures and in experimental animals with genetic defects will be required to evaluate the potential side effects of gene therapy techniques. In our view, solutions to all these problems are needed before any attempt to use gene therapy in human patients could be considered ethically acceptable.

We are aware, however, that physicians have not always waited for a complete evaluation of new and potentially dangerous therapeutic procedures before using them on human beings. Consider how little was known of the basic aspects of virology during Jenner's development of vaccination against smallpox. In this regard, potential gene therapy techniques resemble other medical innovations. There is currently, and there may continue to be, a tendency to use incompletely understood genetic manipulative techniques, borrowed from molecular biology, in clinical settings. We believe that the first attempt at gene therapy in human patients (*8*) illustrates this contention.

The case in question (*8*) concerns two children suffering from hyperargininemia, a hereditary deficiency of the enzyme arginase. The arginase deficiency leads to high concentrations of arginine in the children's blood and cerebrospinal fluid, and has associated with it severe mental retardation (*37*). An attempt has been made to correct this defect at the genetic level by injecting Shope papilloma virus into the children (*8*). The scientific rationale for this treatment is based upon the report that the synthesis of arginase is stimulated in rabbit skin infected with Shope papilloma, and that this new arginase activity had some properties which are different from those of the normal enzyme of rabbit liver (*38*). In 1958, when these experiments were first reported, it was postulated that the viral DNA carried the gene for a viral arginase different from the cellular enzyme. In addition, the serums of laboratory workers who had worked with and

thus been exposed to Shope papilloma virus were tested, and 35 percent of them exhibited lower concentrations of arginine than control hospital patients who had not knowingly been exposed to the virus (*39*). Thus, there were some grounds for believing that inadvertent infection with Shope papilloma in humans could lower the concentration of serum arginine without apparent harmful effects.

More recently, the interpretation that Shope papilloma virus codes for an arginase has been seriously questioned (*40*). It now appears more probable that the virus infection stimulates the production of a cellular arginase. Whether the induced arginase is coded for by viral or by cellular genes is important to the rationale of this attempt at gene therapy. If virus infection induces the synthesis of cellular arginase, and if the children have hereditarily lost the ability to produce arginase, then infecting the children with Shope papilloma virus may not have any possibility of correcting their condition (*41*).

The use of intact viruses as vectors in gene therapy raises further questions. When applied to the skin of rabbits Shope papilloma virus induces skin papillomas, a variable proportion of which develop into cancerous skin lesions. Although Shope papilloma has not had any known harmful effects on humans, tests to establish the safety of large doses have not been performed. It should also be shown that a vector for clinical gene therapy is free from other contaminating viruses latent in the cells used to produce the injected virus.

The clinical results of this therapeutic attempt are not yet known. But we are concerned that this first attempt at gene therapy, which we believe to have been premature, will serve as an impetus for other attempts in the near future. For this reason, we offer the following considerations as a starting point for what we hope will become a widespread discussion of appropriate criteria for the use of genetic manipulative techniques in humans.

Some Preliminary Criteria

We propose the following ethico-scientific criteria which any prospective techniques for gene therapy in human patients should satisfy:

1) There should be adequate biochemical characterization of the prospective patient's genetic disorder. It should be determined whether the patient (i) is producing a mutated, inactive form of the normal protein; (ii) is producing none of the normal protein; or (iii) is producing the normal protein in normal amounts, but the protein is rendered inactive in some way. For example, alterations in membrane structure leading to loss of the cellular receptors for insulin could produce a diabetes-like condition, even though the patient were producing normal amounts of insulin. We anticipate that defects of this type may be found affecting the activity of enzymes which are normally constituents of cell membranes. Our point is that only in the first type of genetic defect (i) would currently envisioned gene therapy techniques be likely to improve the patient's condition.

2) There should be prior experience with untreated cases of what appears to be the same genetic defect so that the natural history of the disease and the efficacy of alternative therapies can be assessed. Thus, the first reported cases of a new human genetic disease would seldom be candidates for attempts at gene therapy. The reason for this criterion comes from our accumulating experience with some of the better studied genetic defects such as phenylketonuria and galactosemia. We now observe heterogeneity in these conditions; that is, what appears to be the same genetic disease turns out to have different genetic bases in different individuals. Widespread screening for phenylketonuria in newborns has detected individuals who, like phenylketonurics, have high concentrations of phenylalanine in the serum just after birth, but have concentrations in the normal range several months later (*2*). It is now also clear that some individuals with high concentrations of phenylalanine in the serum have normal intelligence quotients (*2*). We anticipate that other genetic diseases will exhibit the same kind of heterogeneity. Concern for the welfare of each individual patient dictates that we not rush in with gene therapy until we are very sure about the precise nature and consequences of his genetic defect.

3) There must be an adequate characterization of the quality of the exogenous DNA vector. This will require the development of new, more accurate methods of analyzing the base sequence of the DNA, if synthetic DNA molecules are to be used, or the development of new methods of isolation and purification, if naturally occurring DNA molecules are to be used. We visualize the Food and Drug Administration, or some similar organization, establishing and enforcing quality standards for DNA preparations used in gene therapy.

4) There should be extensive studies in experimental animals to evaluate the therapeutic benefits and adverse side effects of the prospective techniques. These tests should include long-term studies on the possible induction of cancer and genetic disturbances in the offspring of the treated animals. This will require the development of animal models for human genetic diseases. Previous work, which led to the isolation of a mouse strain deficient in the enzyme catalase (*42*) suggests that such animal models could be developed and might yield answers to some of the questions we have raised.

5) For some genetic diseases, the patient's skin fibroblasts grown in vitro reflect the disorder. Thus, in some cases it would be possible to determine whether the prospective gene therapy technique could restore enzyme function in the cells of the prospective patient. This could be done first in vitro, without any of the risks of treating the whole patient. Some side effects, such as chromosome damage and morphological changes suggesting malignancy, could also be assessed at this time. Only when a potential gene therapy technique had satisfied all these safety and efficacy criteria would it be considered for use in human patients.

These criteria omit some other considerations which we believe are important. Although the ethical problems posed by gene therapy are similar in principle to those posed by other experimental medical treatments, we feel that the irreversible and heritable nature of gene therapy means that the tolerable margin of risk and uncertainty in such therapy is reduced. Physicians usually arrive at a judgment regarding the ethical acceptability of an experimental therapy by balancing the risks and consequences of different available treatments against their potential benefits to the patient. In general, the degree of risk tolerated in medical treatment is directly related to the seriousness of the condition.

High-risk treatments are sometimes considered more justified in life-threatening situations. For different human genetic diseases, the severity of the problem in the untreated condition and the response to currently available therapy varies greatly. Thus, phenylketonuria leads to mental retardation, but not death, in most untreated affected individuals, but the mental retardation can be

avoided for the most part by prompt neonatal dietary therapy. In contrast, in the infantile form of Gaucher's disease, a deficiency in the enzyme glucocerebrosidase (important in the metabolism of brain glycolipids) leads to severe and progressive neurologic damage and death within 1 or 2 years (*38*). There is as yet no effective therapy. Thus, the specific characteristics of each genetic disease will be an important factor in evaluating whether or not to attempt gene therapy. We believe that the prospective use of gene therapy will need to be evaluated on a case by case basis.

Another ethical ideal which guides experimental medical treatments is informed consent. By informed consent we mean that the patient, after having the nature of the proposed treatment and its known and suspected risks explained to him by the physician, freely gives the physician his consent to proceed with the treatment. Since many of the cases where gene therapy might be indicated will involve children or newborns as patients, there will be especially troubling problems surrounding informed consent. Parents of newborn children with genetic defects may be asked to give "consent by proxy" for gene therapy. Clearly, until we know much more about the side effects of gene therapy, it will not be possible to provide them with adequate information about risks to the treated individual and his offspring.

Control of Gene Therapy

How can gene therapy in humans be controlled to avoid its misuse? By misuse we mean the premature application of techniques which are inadequately understood and the application of gene therapy for anything other than for the primary benefit of the patient with the genetic disease. In our view, it will be possible to control the procedures used for gene therapy at several levels. For example, between the patient and physician, we can usually rely upon the selection of a therapeutic technique having optimal chances of success. In general, we believe that the doctor will not recommend and the patient will not accept an uncertain, risk-laden gene therapy if a reasonably effective alternative therapy is available. However, the physician, in this as in other cases of experimental therapeutic techniques, has a near monopoly on the relevant facts about risks and benefits of various treatments. Since the physician concerned may also be active in trying to develop the gene therapy technique, how can the patient be protected from a physician who might be overeager to try out his new procedure?

It seems to us that significant opportunities for control also exist at the level of the hospital committees responsible for examining experimental techniques. Already at accredited hospitals, all proposals for research in which human subjects will be used must pass through a review committee. Further control exists through scrutiny of the proposed techniques by the physician's immediate peers.

Procedures to be used for gene therapy might also be controlled by the committees and organizations approving and funding research grants. Moderately large amounts of money will be required for the development of gene therapy techniques, hence there should be competition for public funds with other urgent medical needs. Thus, the first use of gene therapy in human patients would, of necessity, have secured the implied or direct approval of several larger public bodies beyond the principal physician-investigator. In our judgment, these levels of control will probably prove adequate to prevent misuse of projected gene therapy if, as we suspect, gene therapy is attempted in only a small number of instances. Any potential large-scale use of gene therapy (for example, the prospect of treating the approximately 4 million diabetics in the United States with DNA containing the gene for insulin) might appreciably affect the overall quality of the gene pool and would require other forms of control.

Conclusions

In our view, gene therapy may ameliorate some human genetic diseases in the future. For this reason, we believe that research directed at the development of techniques for gene therapy should continue. For the foreseeable future, however, we oppose any further attempts at gene therapy in human patients because (i) our understanding of such basic processes as gene regulation and genetic recombination in human cells is inadequate; (ii) our understanding of the details of the relation between the molecular defect and the disease state is rudimentary for essentially all genetic diseases; and (iii) we have no information on the short-range and long-term side effects of gene therapy. We therefore propose that a sustained effort be made to formulate a complete set of ethico-scientific criteria to guide the development and clinical application of gene therapy techniques. Such an endeavor could go a long way toward ensuring that gene therapy is used in humans only in those instances where it will prove beneficial, and toward preventing its misuse through premature application.

Two recent papers have provided new demonstrations of directed genetic modification of mammalian cells. Munyon *et al.* (*44*) restored the ability to synthesize the enzyme thymidine kinase to thymidine kinase–deficient mouse cells by infection with ultraviolet-irradiated herpes simplex virus. In their experiments the DNA from herpes simplex virus, which contains a gene coding for thymidine kinase, may have formed a hereditable association with the mouse cells. Merril *et al.* (*45*) reported that treatment of fibroblasts from patients with galactosemia with exogenous DNA caused increased activity of a missing enzyme, α-D-galactose-1-phosphate uridyltransferase. They also provided some evidence that the change persisted after subculturing the treated cells. If this latter report can be confirmed, the feasibility of directed genetic modification of human cells would be clearly demonstrated, considerably enhancing the technical prospects for gene therapy.

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Protein Synthesis: Its Control in Erythropoiesis

Cellular differentiation is explored in the erythroid cell system of the mouse.

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The regulation of mammalian cell differentiation remains one of the major unresolved problems in biology. Operationally, the definition of the differentiated state generally involves the capacity for synthesis of specialized proteins. To elucidate the mechanisms controlling the initiation of synthesis of differentiated proteins one must study the precursor of the differentiated cell. In almost all mammalian cell populations there is heterogeneity with regard to the stage of differentiation and with regard to the type of differentiated cell. To identify the initiating event in the onset of synthesis of differentiated protein of a given cell line, the precursor cell must be isolated for quantitative, analytical studies. In the total cell population of most differentiating mammalian cell systems such cells are present in a very low proportion.

In erythroid cell systems a substantial beginning has been made in the analysis of aspects of the regulation of the biologic activity of the precursor cell (*1, 2*). Erythroid cells synthesize a predominant type of protein, the hemoglobins. There is a considerable body of knowledge with respect to the genetic, chemical, physiological, and cytological aspects of erythroid cells. The hormone, erythropoietin, has a role in inducing erythropoiesis and hemoglobin synthesis. While no single mammalian cell system is ideal for exploring the full range of problems that are critical to the understanding of the regulation of protein synthesis in differentiating cells, the erythroid cell system has provided considerable insight into a number of these, such as: (i) the cellular basis for changing patterns of hemoglobin synthesis during fetal and postfetal development; (ii) the relationship between primitive and definitive erythroid cell lines; (iii) the mitotic activity of erythroblast and the relationship to hemoglobin synthesis; (iv) the stability of messenger RNA in relation to the synthesis of different classes of proteins during erythroid cell differentiation; and (v) the nature of erythropoietin action on cell differentiation and hemoglobin synthesis.

Patterns of Hemoglobin Synthesis

Changes in the types of hemoglobins synthesized during fetal and postfetal development have been described for the mouse, man, tadpole, chick, and a variety of other species (*3*). Studies in the mouse have provided perhaps the most direct evidence on the relationship between alterations in the cell line active in erythropoiesis and the type of hemoglobin formed (*4*). In the mouse, whose gestation period is 21 days, the first morphologically identifiable site of erythropoiesis occurs in the blood islands of the yolk sac at approximately the eighth day of gestation (*1*). Proliferation of erythroid cell precursors is active in these sites from about the eighth to the tenth days (*1, 5*). These cells enter the fetal circulation as nucleated erythroblasts by the ninth day of gestation. From the tenth day onward, further division and differentiation of these cells proceeds in the circulation. Mitosis may be observed through day 13 of gestation. Ribosomes and polyribosomes, abun-

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