Human Gene Therapy

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Human Gene Therapy

Background Paper

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"Where two principles really do meet which cannot be reconciled with one another, then each man declares the other a fool and heretic. "

–Ludwig Wittgenstein, 1950-1951

"Even in the extreme case where disagreement extends irreducibly to ultimate moral ends, the proper counsel is not one of pluralistic tolerance . . . We can still call the good good and the bad bad, and hope . . . that these epithets may work their emotive weal."

"Thus we do what we can with our ultimate values, but we have to deplore the irreparable lack of the empirical checkpoints that are the solace of the scientist. Loose ends are untidy at best, and disturbingly so when the ultimate good is at stake."

—Willard Van Orman Quine, 1981

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Preface

This background paper is the fourth in a series of OTA publications on genetics, and the third in a series focusing on emerging biological technologies. * It was prepared at the request of Representative Albert Gore, Jr., as Chairman of the Subcommittee on Investigations and Oversight of the Committee on Science and Technology, U.S. House of Representatives. Preparation of the paper involved extensive assistance from and review by experts and other interested parties (apps. C and D), and included a workshop convened at OTA on September 25, 1984.

Interest in human applications of recombinant DNA technology has been expressed by numerous scientific, medical, religious, civic, and government leaders by Representative Gore's subcommittee and resulted in congressional hearings in November 1982, Human gene therapy is currently preeminent among the the topics of concern. This paper focuses on the imminent development of human gene therapy, emphasizing early medical applications. The governmental concerns related to human gene therapy, as for other medical technologies, will include protection of subjects involved in research and clinical treatment, ensuring safety and efficacy of the techniques in specific applications, and public discussion and education.

Human gene therapy, if it is approved for use, will first be performed on patients who have no better prospect for treatment, and who suffer from severe, rapidly fatal diseases caused by defective genes. Treatment will involve inserting copies of the normal gene into cells where the new gene can be used to produce proteins that correct a biochemical defect. Human gene therapy as currently envisioned would thus be applied to treat patients with specific rare genetic diseases, and not as the tool of a eugenic social program intended to improve the human gene pool.

Gene therapy in humans will first be done in cells from an organ or tissue other than germ cells, probably from a patient's bone marrow. Such treatment would therefore not lead to heritable changes. Therefore, because cells that are used in reproduction are not involved, gene therapy of this type is quite similar to other kinds of medical therapy, and does not pose new kinds of risks. When considering gene therapy that does not result in inherited change, the factor that most distinguishes it from other medical technologies is its conspicuousness in the public eye; otherwise it can be viewed as simply another tool to help individuals overcome an illness.

Public interest in gene therapy suggests that utmost care must be taken to ensure that the process for approving its early application is fair, open, and thorough. Several Federal agencies, including the Recombinant DNA Advisory Committee at the National Institutes of Health and the Food and Drug Administration, are presently involved in just this process.

It is generally agreed that gene therapy that affects only the patient is analogous to other medical technologies. There is, however, no agreement about the need, technical feasibility, or ethical acceptability of gene therapy that leads to inherited changes. Commencement of gene therapy that would involve inherited changes should not proceed without substantial further evaluation and public discussion.

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*The other OTA publications on genetics are ***Impacts of Applied Genetics*** (April 1981), ***The Role of Genetic Testing in the Prevention of Occupational Disease*** (April 1983), and ***Commercial Biotechnology: An International Analysis*** (January 1984). The other publications on novel biological technologies are ***Impacts of Applied Genetics*** and ***Impacts of Neuroscience*** (March 1984).

Advisory Panel for OTA Workshop on Human Gene Therapy, Sept. 25, 1984

LeRoy Walters, Chairman,
Director, Center for Bioethics
Kennedy Institute of Ethics, Georgetown University

Non-Government Panelists

Lori Andrews
American Bar Foundation

James E. Bowman
Professor of Pathology and Medicine, and
Committee on Genetics
University of Chicago

C. Thomas Caskey
Howard Hughes Institute Medical Investigator
and Professor of Medicine
Baylor College of Medicine

Theodore Friedmann
Professor of Pediatrics
School of Medicine
University of California, San Diego

Ola Huntley
Board of Directors
Sickle Cell Self-Help

Horace Judson
Henry R. Luce Professor
Writing Seminars
Johns Hopkins University

J. Robert Nelson
Professor of Theology
Boston University

Nanette Newell
Calgene, Inc.

Albert Rosenfeld
Consultant on Future Programs
March of Dimes

Seymour Siegel
Ralph Simon Professor of Ethics and Theology
Jewish Theological Seminary of America, and
Executive Director
U.S. Holocaust Memorial Council

Carol Struckmeyer
Genetic Associate and Program Coordinator
New Hampshire Genetic Services Program

Government Panelists

W. French Anderson
Chief, Laboratory of Molecular Hematology
National Heart, Lung, and Blood Institute

John C. Fletcher
Assistant for Bioethics
Warren G. Magnuson Clinical Center
National Institutes of Health

Elaine Esber
Director, Office of Biologics Research and
Review
Food and Drug Administration

Judith A. Johnson
Analyst in Life Sciences
Science Policy Research Division
Congressional Research Service
Library of Congress

Henry Miller
Medical Officer
National Center for Drugs and Biologics
Food and Drug Administration

Robert Nicholas
Staff Director
Subcommittee on Investigations and Oversight
Committee on Science and Technology
U.S. House of Representatives

OTA Project Staff for Human Gene Therapy

Roger C. Herdman, ***Assistant Director, OTA***
Health and Life Sciences Division

Gretchen S. Kolsrud, ***Biological Applications Program Manager***

Robert M. Cook-Deegan, ***Study Director, Senior Analyst***

Teresa Schwab,[1] ***Research Analyst***

L. Val Giddings,[1] ***Analyst***

Nanette Newell,[2] ***Senior Analyst***

Eleanor Pitts,[3] ***Research Assistant***

Stephen K. Eckman,[4] 1984 ***Wharton Public Policy Fellow***

Contractor

Lawrence Wissow, ***Johns Hopkins University***

Support Staff

Sharon Smith, ' ***Administrative Assistant***

Elma Rubright,[3] ***Administrative Assistant***

Linda Ray ford, ***Word Processing Specialist***

[1]Since September 1984
[2]Until April 1984.
[3]Until August 1984
[4]June to August 1984

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HUMAN GENE THERAPY

Advances in molecular biology have triggered an unprecedented expansion of knowledge about human genetics. The rise of new genetic technologies, and their implied power, has engendered concerns among religious, scientific, and civic leaders that these new technologies may be growing more rapidly than our ability to prudently control and productively use them. The ability to insert human genes into human patients to treat specific genetic diseases–human gene therapy–has been one of the concerns noted by those observing the evolution of genetic technologies.[1]

Human gene therapy will first be considered in a clinical situation where it might be possible to treat with a human gene an individual patient suffering from a genetic disease. Gene therapy would be attempted only when there is no other therapeutic alternative, or when the alternatives are judged to be of greater risk or less potential benefit. Application of gene therapy for a human genetic disease should require evidence that it is safe, might prove beneficial, is technically possible, and is ethically acceptable. Judgments should be made in a procedurally sound and objective regulatory framework.

Some of the concern about the potential abuse of gene therapy may be allayed by considering the following points:

- The most promising prospects for human gene therapy involve treatment of specific genetic diseases by methods that are not designed to cause inherited changes, and the ethical concerns may thus be similar to those associated with other medical technologies, such as vaccination or drug administration, currently in use (President's Commission, 1983; Shinn, 1982; Fletcher, 1982, 1983; Siegel, 1982, 1983).
- The capability for human gene therapy will almost certainly develop in small increments, like other medical technologies. This likelihood, combined with the lack of inheritance of anticipated genetic alterations, suggests that decisions to proceed will not lead to irreversible population effects.
- Inherited alterations, the most controversial potential applications of gene therapy, are unlikely to be undertaken in humans in the near future because they are technically too difficult, are perceived as ethically problematic, and may not prove superior to existing technologies.
- There is a regulatory framework already in place for considering the first applications of human gene therapy. The existence of established procedures cannot guarantee that they will be followed, because some scientists or physicians may choose to deviate from them, but there are laws in place that can be enforced. The existence of such a regulatory framework distinguishes gene therapy from many other novel biological technologies.

The primary justification for attempting human gene therapy is the number and severity of genetic diseases. There are 2,000 to 3,000 known genetic diseases—i.e., diseases whose roots can be traced to specific genes or known inheritance patterns (McKusick, 1983), As many as 2 percent of newborn infants suffer from a genetic disease (Lubs, 1977). For most such diseases, the defective genes have not been identified or located. For several, including some of the most severe childhood diseases, the gene that causes the disease ***has*** been found, and for a few such diseases, copies of the normal gene are available through use of recombinant DNA technology. Human gene therapy will be feasible only for those diseases in which the defect has been identified and the normal gene has been isolated and cloned. All of the diseases presently under consideration for gene therapy are rare.

Gene transfer experiments in animals have produced some inherited changes, but the ethical questions and relative inefficiency of ***current*** techniques preclude application to humans. Because most of the serious concerns that human gene therapy might cause long-term changes in human populations presume ***inheritance*** of characteris-

[1]The development of recombinant DNA and other advanced techniques of molecular biology have permitted novel applications of biological methods in industry and health care through the new biotechnology. This background paper is not about industrial or medical applications of biotechnology, but rather about deliberately changing genetic information in humans.

tics, the present state of the technology does not pose fundamentally new ethical problems. Human gene therapy that does lead to inherited changes, however, would likely incite deep-seated apprehensions about premature application. There should be ample opportunity for public discussion before germ line gene therapy is tested in humans. The body of this background paper will explicate these statements by surveying the technical prospects for human gene therapy and discussing the public policy considerations.

Direct genetic alterations have been successfully practiced in bacteria, yeasts, fruit flies, and some mammals. To date, scientists have not succeeded in applying these same techniques to correct the action of defective genes or directly to change the genome of a human being. The barriers to correcting the genetic defects that cause a few human diseases, however, are now primarily technical, and these barriers may be overcome within the next few years. There are already grant applications to the National Institutes of Health that could lead to clinical testing of human gene therapy. Requests for permission to begin the actual clinical research that would involve humans have not, however, been received to date.

"Human gene therapy," for the purposes of this report, refers to the deliberate administration of genetic material into a human patient with the intent of correcting a specific genetic defect. This would include, for example, replacement of the defective gene in bone marrow cells of a child affected by genetic immune deficiency. Most discussion in this background paper centers on noninherited gene therapy because it is the type expected to be considered soon.

Gene therapy, as defined here, would not include genetically enhancing general characteristics such as behavior, intelligence, or physical appearance. These are excluded from the definition, although the prospects for influencing such traits in the population through genetic methods are discussed in some sections because concern about such prospects has been raised in public debate (Subcommittee on Investigations and Oversight, 1982; Siegel, 1983; Rifkin, 1983; Foundation on Economic Trends, 1984; National Council of Churches, 1984; World Council of Churches, 1983). Enhancement of complex human traits may never be practical or socially accepted and it is not "therapy" for a specific disease.

The definition used in this report thus focuses on correction of specific genetic defects in individual patients, except when social concerns about other applications or general issues are explicitly recognized. This background paper summarizes the technical, medical, and social considerations that arise from consideration of genetic manipulation in humans and how they relate to Federal policy.[2]

[2]Genetic technologies that do not involve gene *therapy*, including agricultural, pharmaceutical, and other industrial applications, have been discussed in several earlier reports issued by the Office of Technology Assessment (OTA) of the U.S Congress. ***Impacts of Applied Genetics,*** issued in 1981, dealt with non-human applications of biotechnology *The Role of" Genetic Testing in the Prevention of' Occupational Disease,* issued in 1983, covered the use of genetic screening in the workplace; and ***Commercial Biotechnology: An International analysis,*** issued in January 1984, surveyed and analyzed the commercial development of biotechnology in Japan Western Europe, and the United States. Issues and topics considered in these other OTA publications are not repeated here; rather, this background paper explores new issues relating to gene therapy in humans.

Why is Congress interested in human gene therapy now?

Congressional interest in human gene therapy stems from general awareness of the rapid progress in molecular genetics combined with concern about the potential power and impact of new biological technologies. Some believe that the deliberate "engineering" of humans who are physically or intellectually "superior" is morally repugnant or politically dangerous, and there is fear that the new techniques might be used to attempt such engineering (Rifkin, 1983; Foundation on

Economic Trends, 1984). Human gene therapy that leads to inherited changes, in particular, has been identified as a "fundamental concern for the protection of the integrity, value, and health of human life, both of individuals and of large numbers. The putative possibility of performing germ line therapy, however noble in intention, would incur risks of unknown magnitude to future progeny" (Nelson, 1984b). Several events contributing to the public interest in molecular genetics are of particular interest.

History

In 1972, scientists joined DNA fragments from two species, resulting in the first deliberately created recombinant DNA molecule (rDNA) (see Technical Notes 1 and 2 for further details). In 1973, rDNA molecules were first duplicated and grown in bacteria. Concern about the safety of recombinant DNA laboratory research led scientists to call for a worldwide moratorium on certain types of experiments. Several scientific and political meetings, some of them quite contentious, were held that focused on issues of safety (Wade, 1984). In 1974, the Recombinant DNA Advisory Committee (RAC) was formed to advise the National Institutes of Health in formulating guidelines for research; the first guidelines were issued in 1976 (Milewski, 1984).

Commercial interest in biotechnology became evident in 1976 when the first new firm, Genentech, was established specifically to apply recombinant DNA technology to medicine and other areas. Since that time, more than 200 firms have been founded to exploit the new technologies (Office of Technology Assessment, 1984). Two patent decisions in 1980 highlighted the commercial potential of new biological technologies. In one, a bacterial strain was patented that had been developed using traditional methods of selecting for genetic traits, and without resort to recombinant DNA technology.3 This was the first patent issued for a living organism. The second patent was issued for the technique of making certain types of recombinant DNA molecules.[4]

Wall Street responded to the promise of biotechnology in 1981 by setting a record for the fastest price-per-share increase when Genentech's initial public offering of stock rose from $35 to $89 per share in 20 minutes. Optimism was again noted in 1982 when Cetus made a large and successful initial public offering ($115 million). Early commercial expectations were encouraged when the first commercial product using recombinant DNA technology was introduced to the market in 1982: human insulin, sold as Humulin (Office of Technology Assessment, 1984, ch. 4). Many of the expectations of rapid economic bonanza have been tempered by the length of time and magnitude of effort required to bring products to the market, but long-term prospects for commercial applications of biotechnology remain promising (Office of Technology Assessment, 1984).

Developments in regulation, law, and finance were attended by continued advances in genetic research. The surprising discovery of "split" genes occurred through the use of recombinant DNA technologies in 1977.[5] That same year, two independent techniques were developed for determining the DNA sequences that contain genetic information, permitting direct inspection of the genetic material and analysis of its functions (Watson, 1984).

Advances in medical applications also occurred. Recombinant DNA techniques were first used for the prenatal detection of sickle cell disease in 1982 (Chang and Kan, 1982; Orkin, Little and Kazazian, 1982). Use of enzymes that specifically cut DNA, in combination with probes that detect specific

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[3]The first patent for a microorganism was granted to Ananda Chakrabarty of the General Electric Corp. for a strain of Pseudomonas bacterium that digests certain petrochemicals. Dr Chakrabarty developed the strain by growing rare and mutant forms of the bacteria in new artificial environments until a strain with the desired characteristics resulted. The decision to grant the patent was made by the U.S. Supreme Court in a 5 to 4 vote on June 16, 1980

[4]This patent was granted to Stanley Cohen of Stanford University and Herbert Boyer of the [University of California at San Francisco for the basic process of constructing recombinant DNA molecules. The patent is questioned by some, but has not been seriously challenged at the time this is written (Office of Technology Assessment, 1984, ch. 16). The patent has since been complemented by a process patent for the same technology that was granted in August 1984.

[5]Scientists confirmed their expectations that the genes were more complicated in higher animals rompared to bacteria. Genes in higher organisms are often divided into regions: the sequence for a protein, for example, may be separated into several units, and the units must be rearranged and "spliced" together to form the sequence that is eventually used to produce the protein (Leder, 1978).

sequences of DNA, led to development of a method for determining the location of genes, even when their function had not been determined and the genes had not been isolated (Botstein, 1980; Botstein, 1984). The technique, first described in 1980, has great promise for both promoting understanding of human genetics and assisting in the diagnosis of hereditary diseases (see app. A). In 1980, the first inherited alteration of genes in the germ line of mice was achieved (Gordon and Ruddle, 1981) and in 1982, the gene for rat growth hormone was introduced into mice (Palmiter, 1982, 1983). The mice that incorporated the rat growth hormone genes into their cells could be induced, using a special diet containing zinc, to grow to twice normal size. The response to zinc was due to a special DNA sequence that the scientists had included with the growth hormone gene that caused zinc to "turn on" the inserted gene. The progeny of the genetically altered mice also inherited the new foreign gene, making them "mighty mice" as well.

The human experiments of Martin Cline, a physician from the University of California at Los Angeles, contributed to the ethical apprehensions of many observers. Dr. Cline attempted gene therapy using recombinant DNA in two patients suffering from thalassemia, a disease causing severe anemia (see Technical Note 5)—one in Israel and one in Italy. The propriety of the experiments was widely questioned in the scientific literature (Wade, 1980; Wade, 1981). Many scientists and clinicians judged the human experiments premature (Fletcher, 1982a, 1982b; Anderson, 1982) and pointed out that Dr. Cline did not even follow the protocol that had been approved by the foreign human subjects review boards. He also failed to wait for approval by such committees in the United States (Talbot, 1982). Professor Cline was penalized by the National Institutes of Health by termination of two grants, and he resigned chairmanship of his division at the University of California (Sun, 1981, 1982; Talbot, 1982). (Dr. Cline's experiments and the dispute over their propriety are described in greater detail below.) The history of human gene therapy thus did not have an auspicious start, although many scientists and clinicians would not consider Dr. Cline's experiments bona fide attempts at human gene therapy.

Concern among religious leaders

Increasing commercial interest, progressive movement of the technology into the relatively unregulated private sector, possible premature applications to humans, and impressive technical improvements all attracted attention to molecular genetics in the early 1980s. The general secretaries of three large religious bodies—the U.S. Catholic Conference, the Synagogue Council of America, and the National Council of Churches—sent a letter to President Carter in 1980 in which they expressed concern that prowess might surpass prudence in the human application of genetic technologies. They noted that we had entered an "era of fundamental danger triggered by the rapid growth of genetic engineering)" and appealed to the President to look into how molecular genetics might be applied to humans (President's Commission, 1982, pp. 95-96). The letter noted that there was no governmental agency or committee investigating the ethical, social, and religious questions raised by the new technologies. Such questions included concern for fair distribution of risks and benefits, control of genetic experimentation, and long-term consequences of genetic interventions.

The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research (hereafter called the "President's Commission") responded by investigating some uses of recombinant DNA in humans. The Commission's inquiry resulted in publication of Splicing Life in November of 1982 (President's Commission, 1982). In that same month, the Subcommittee on Investigations and Oversight of the Committee on Science and Technology, U.S. House of Representatives, held hearings for 3 days entitled ***Human Genetic Engineering.***

A resolution signed by 56 religious leaders and 8 scientists and ethicists rekindled interest in human genetics when it was sent to Congress in June of 1983 and introduced by Senator Mark O. Hatfield ***(Congressional Record,*** June 10, 1983, S 8202-8205). The resolution urged that '(efforts to engineer specific genetic traits into the germ line of the human species should not be attempted" (reprinted in: Foundation on Economic Trends, 1984). The signatories of the resolution came from

a broad spectrum of political and religious viewpoints, including diverse Protestant, Roman Catholic, and Jewish representatives (signatories and resolution printed in: Recombinant DNA Advisory Committee, 1984). The resolution was accompanied by a discussion paper by Jeremy Rifkin, author of ***Algeny*** and head of the Foundation on Economic Trends, although the discussion paper was not endorsed by all signatories of the resolution (Nelson, 1984a; McCormick, 1984; Dorfman, 1983). The discussion paper warned of many potential abuses of intervening in human genetics (Foundation on Economic Trends, 1984; Recombinant DNA Advisory Committee, 1984). Delivery of the resolution, and the involvement of Rifkin and many of the signers attracted media attention, once again verifying the existence of public and religious apprehensions about the rapid advances of genetic technologies (Harden, 1984). Discussions following release of the resolution have failed to demonstrate a consensus, even among the signatories, but the document did generate the wide public discussion sought by many who signed it (Nelson, 1984a; McCormick, 1984).

OTA involvement and review process

OTA convened a workshop in September 1984, where potential consumers and experts in ethics, medicine, and genetics convened to discuss the technical feasibility and diverse implications of human gene therapy. The panel for the workshop and other workshop participants reviewed material prepared by OTA staff and contractors. Several drafts of the background paper were widely circulated for external criticism before and after the workshop, resulting in review by more than 70 ethicists, scientists, religious and civic leaders, and other concerned parties. Drafts were also distributed for review at the National Institutes of Health, the Food and Drug Administration, to all members of the Working Group on Human Gene Therapy of the Recombinant DNA Advisory Committee of the National Institutes of Health, and to other government agencies.

Types of gene therapy

Human gene therapy encompasses a broad range of technologies and may eventually be applied to a diverse group of genetic diseases. This variety requires that several distinctions be kept in mind when discussing the technology.

Different mechanisms of gene therapy

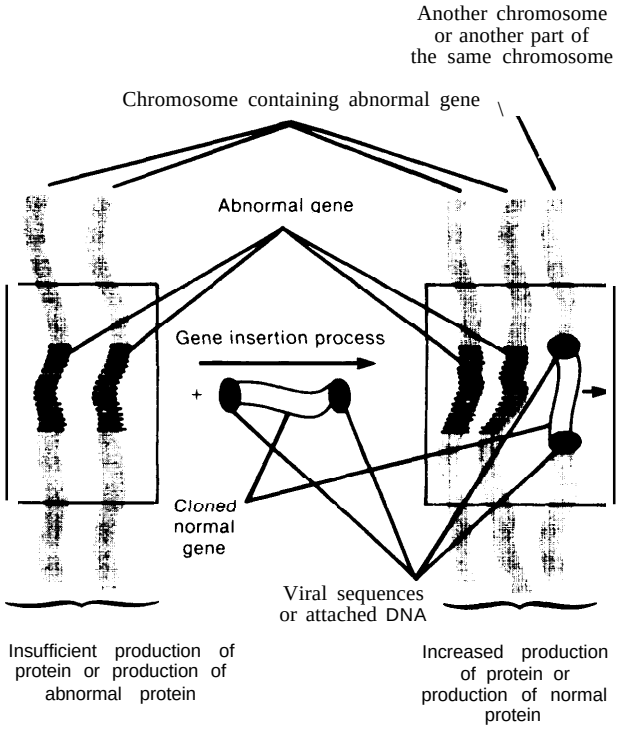
Gene therapy refers to the insertion of genetic material to correct a defect. Gene therapy can take several forms:

- gene insertion, in which a new version of a gene is introduced into a cell;
- gene modification, in which a gene already in place is altered; and
- gene surgery, in which a particular gene is excised and may also be replaced by its normal counterpart.

Such genetic alterations would involve insertion of new material that directly codes for proteins or that affects how existing genes are expressed by suppressing or enhancing production of particular proteins.

Current prospects for human gene therapy do not include either gene modification or gene surgery (Anderson, 1984) because these are more complex than merely adding new genes to cells, Such complicated manipulations can now be performed, however, in some viruses, yeast, and bacteria, and the necessary technologies may later be discovered that would permit gene surgery or controlled genetic modification in animals and humans. Through the remainder of this background paper, gene therapy will refer to gene insertion, because this is the form likely to be applied first. The distinction is technically relevant,

Gene Insertion



SOURCE: Office of Technology Assessment.

but does not significantly affect the discussion of public policy implications that will be addressed because gene modification and gene surgery do not raise moral or medical issues distinct from those raised by gene insertion.

Somatic versus germ line gene therapy

Gene therapy might be performed in either **germ cells** (sperm, egg cells, or the cells that give rise to them) or in ***somatic*** cells (cells that comprise all other body tissues). Alterations in somatic cells do not result in inheritance of the alteration, while modification of germ cells results in changes that could be passed on to subsequent generations if the recipient patient were to have children.

Genes are comprised of deoxyribonucleic acid (DNA). DNA, in turn, is composed of long chains of molecules called nucleotides. All the genetic information that is inherited by a cell is encoded by the sequence of nucleotides in its DNA (see Technical Notes). DNA ultimately controls formation of all of the substances that comprise and regulate the cell. Certain sequences of DNA contain information for specific proteins such as enzymes, hemoglobin (the oxygen-containing protein in red blood cells), or the variety of receptors on the cell's surface. Stretches of DNA that contain the information for a specific product are called genes. The DNA of the gene would not be different for somatic versus germ line therapy, although there might be different sequences added adjacent to the gene depending on how the gene would be regulated in a particular experiment or treatment. The difference between somatic and germ line therapy is which type of cell is treated with DNA.

Somatic cell gene therapy is illustrated by following how physicians might attempt to correct the genetic defects that cause ADA or PNP enzyme deficiencies. ADA deficiency is caused by absence or inactivity of the enzyme adenosine deaminase. PNP deficiency is a different disorder with some clinical similarities, It is caused by absence or inactivity of the purine nucleoside phosphorylase enzyme. In ADA deficiency, the DNA in the adenosine deaminase gene is abnormal, and for PNP deficiency, there is a corresponding defective PNP gene. The genetic defect is due to an incorrect DNA sequence caused by a mutation. The mutation could be in the form of errant replacement of one nucleotide by another or loss (or addition) of one or more nucleotides somewhere in the sequence. The altered sequence encodes an abnormal enzyme that does not function, or causes insufficient production of the normal protein.

Because there is either not enough enzyme, or it is present in a dysfunctional form, the chemical reactions mediated by ADA or PNP do not take place normally in the cell. This leads to accumulation of some chemicals that would normally be destroyed by ADA or PNP, and a paucity of those chemicals the enzymes are responsible for making. In the case of both ADA and PNP deficiencies, it appears that toxic chemicals accumulate that inhibit the action of cells that are involved in body defences.

The diseases are inherited as recessive genetic traits (the two diseases caused by the different enzyme deficiencies are slightly different, but not in a sense that is relevant here), and are usually fatal before age 2 if not treated (Kredich, 1983).

Severe immune deficiencies can be treated by bone marrow transplant (Friedrich, 1984), but not all patients are eligible for transplant, and the procedure is quite risky and costly. ADA or PNP deficiency might be treated instead by somatic cell gene therapy: removing an affected patient's bone marrow cells, inserting normal genes for the enzymes into them, and returning the treated cells to the patient where they could grow and perhaps produce enough of the needed enzyme to degrade the toxic chemicals, thus restoring immune function.

Although the details vary, most of the diseases that might be approached by gene therapy conform to this model: they are genetic defects that cause insufficient production of normal enzymes or production of dysfunctional ones. Gene therapy attempts to restore enzyme function by inserting DNA to produce normal protein.

Rather than treating only bone marrow or other somatic cells, germ cells or cells of an early embryo might be treated to correct a genetic defect. Such germ line treatment would affect all cells in the body, including both somatic cells and germ line cells. In the case of ADA or PNP deficiency, germ line therapy would likely be done by inserting the correct genes into an affected embryo within hours of fertilization. This might lead to presence of a normal ADA or PNP gene in all cells, and expression of the normal gene with production of a normal enzyme in the tissues where it would be needed to correct the immune deficiency.

In somatic cell therapy, treatment affects only cells in the patients' organs and would not be passed on to children, while germ line correction would produce genetic changes that could be detected in all cells in the body and could be passed on to children.

TREATMENT OF SOMATIC CELLS

Many of the ethical and religious reservations expressed about human gene therapy refer only to alterations that might affect the germ line to produce inherited changes, In the opinion of several ethicists and religious thinkers, treatment of somatic cells by genetic methods does not pose ethical problems different in kind from those presented by other types of experimental therapy such as new drugs or novel surgical techniques (Fletcher, 1983a, 1983b; Siegel, 1982, 1983). The questions that need to be addressed in assessing the appropriateness of treating ***somatic*** cells include:

- What is the likely impact on people's *regard* for the sanctity of human life? (World Council of Churches, 1983; National Council of Churches, 1984).
- What are the risks of inadvertently affecting the germ line?
- What are the precautions taken against deliberate misapplication?
- What scientific data are available to suggest that the treatment might work to the patient's benefit?
- How serious is the disease? What are the realistic possibilities of benefit to the patient? What are the risks to the patient? What is the prognosis if there is no treatment?
- What are the alternative methods of treatment? Is gene therapy likely to be more effective, less costly, safer, or otherwise more acceptable than available alternatives?
- How safe is the procedure, based on the best available evidence? What are the data on short-term effects and long-term consequences?
- Are patients or their surrogate decisionmakers properly informed about the risks and benefits of the therapy?
- Are the side effects of the treatment reversible or treatable in the patient and in the population?

These concerns are analogous to those that would be raised for any other new medical treatment. The likelihood of inadvertently affecting the germ line, however, is of greater concern for gene therapy than for most other treatments. The risk of genetically altering the germ line is not unique to gene therapy because several other medical practices —such as vaccination, cancer chemotherapy and radiation therapy—also carry this risk (see "Safety" below).

A concern for deliberate misapplication of gene therapy derives, in part, from a historic association between eugenics and oppressive political movements (see below). Genetic "purity" or preservation of "superior" characteristics by genetic

means has been advocated by several political and scientific groups in the past (Kevles, 1984), and some fear that gene therapy technology might become part of a coercive social program. The rationale for gene therapy as currently contemplated–insertion of single genes to correct severely debilitating specific genetic diseases (Anderson, 1984)—is extremely remote from such eugenic motivations.

The question regarding the sanctity of human life is one that has been addressed by religious thinkers and philosophers (Siegel, 1982, 1983; President's Commission, 1982). This concern for human dignity underlies the great care with which proposals to undertake human gene therapy are now being scrutinized. Such concern suggests that public education and discussion must precede and attend clinical application (Working Group on Human Gene Therapy, 1984; President's Commission, 1982; Capron, 1984a,b).

TREATMENT OF GERM CELLS

If ever applied to humans, germ line therapy could be done in several ways. Such therapy could be directed at sperm or ova, or cells that produce them, before the germ cells join to produce a fertilized egg. It could also be targeted at the early stages of development, currently practical only if performed within hours after fertilization, days before the embryo is implanted in the uterus.[6] Human gene therapy affecting germ line cells raises several concerns in addition to those listed for somatic cell therapy. These have been noted by religious and civic commentators (Foundation on Economic Trends, 1984; National Council of Churches, 1984; President's Commission, 1982), and include:

- propagation of unpredictable effects (both positive and negative) into future generations,
- diminishing genetic diversity among human populations, and
- long-term effects of changing genetic characteristics in human populations.

The different social and ethical considerations that arise from somatic versus germ cell manipulations are elaborated further in the sections below on medical and social aspects of gene therapy.

COMPARISON OF SOMATIC AND GERM CELL GENE THERAPY

There are several technical and practical advantages to performing gene therapy on somatic cells as opposed to germ cells. The primary advantage of somatic cell therapy is that it can be performed on individuals at any stage of development, while germ line therapy as currently envisioned would have to be performed early in embryonic development. Experiments on somatic cells may be done on samples or parts of organs, rather than an entire organ, lowering the risks of failure because a failed experiment does not cause loss of the organ. Experiments involving somatic cells may also be repeated in the same individual if they fail, and the reliability of the gene transfer procedure does not have to be as high. Somatic cell gene therapy is also advantageous because it directly benefits the person to whom it is administered, rather than a person (who cannot consent to therapy) who develops from a treated embryo.

Despite these advantages of somatic therapy, there are several disadvantages. Somatic cell therapy may not be applicable to some disorders that affect multiple tissues, because cells of each organ would have to be altered. It may also not be effective for those tissues composed of cells that do not divide, such as brain and muscle (although symptoms of some diseases of nerve and muscle cells might be treated by gene therapy in other kinds of cells that influence brain and muscular function). Which diseases and which tissues might prove refractory to gene therapy of somatic cells will be determined only by further study of the specific genetic diseases in question.

There is at least one potential advantage to heritable correction of germ line cells. Once a defect were fixed, it would be less likely to plague the direct descendants of the person who developed from the treated embryo. This would not eliminate the risk, however, because new mutations causing the same disease could spontaneously arise.

[6] For further details on stages of fertilization and human development, see Technical Notes.

TREATMENT OF SPERM, OVA, AND CELLS THAT PRODUCE THEM

While germ line therapy, until now, has been performed on early embryonic cells, it is theoretically possible to perform it by inserting new genetic information into gametes (sperm, ova, or the cells that produce them).

Sperm may be difficult to genetically alter, because they are small, difficult to penetrate by physical or chemical manipulations, and would have to be treated in vast numbers. Millions of sperm are usually inseminated before fertilization, although only one actually fertilizes the egg; every sperm would have to be treated if gene therapy were to be assured. It would be technically easier to genetically alter sperm by treating the cells that produce them because such cells are larger and less difficult to manipulate. There are several complications with this strategy, however, including the necessity to use invasive procedures to obtain testicular cells, unavailability of methods for artificially inducing maturation of sperm, and uncertainty over whether genetic changes in sperm precursors would lead to genetic correction in all sperm. Substantial technological advances would thus be required for reliable gene therapy of sperm or their precursor cells.

In contrast, ova, or egg cells, might be altered after they were extruded from the ovary, and before fertilization. Egg cells are larger and more easily manipulated than sperm, suggesting that eggs might be easier candidates for gene insertion. Methods for obtaining human ova are now routinely practiced for in vitro fertilization techniques, and many do not involve highly invasive techniques (Andrews, 1984c). Manipulations of egg cells and early embryos differ primarily in that the eggs could be altered before fertilization, eliminating some ethical concerns of those who regard fertilization as the beginning of human life. Unless the gene therapy technique were extremely reliable, however, methods would have to be found for confirming that the desired alterations had actually occurred. This would involve sampling of embryonic or fetal tissue, and would thus not avoid all of the ethical questions that beset embryonic manipulations.

Gene therapy of gametes thus offers some advantages in restricted applications, but it would affect the germ line, and would not avoid the ethical dilemma associated with heritability of genetic changes. The technical prospects for such therapy, however, are less promising than treatment of either early embryos or somatic cells. For both technical and ethical reasons, therefore, gametic gene therapy is not imminent.

IN VITRO VERSUS IN VIVO

Gene therapy can theoretically be performed either on cells that have been removed from the body (in vitro), or on cells that are in their usual place in the body (in vivo). The first attempts at human gene therapy will be performed on cells that are removed from the body, genetically altered in vitro, and restored to the patient, as in the example of ADA or PNP deficiencies (Anderson, 1984). This procedure makes the chances of altering the germ line of the patient quite low, and also reduces the probability of unintentionally affecting other tissues that need not be treated (Working Group on Human Gene Therapy, 1984).

Several disorders in addition to ADA and PNP deficiencies are currently under discussion for somatic cell gene therapy. Citrullinemia is caused by deficiency of the enzyme arginosuccinate synthetase involved in protein and amino acid metabolism and nitrogen excretion (Walser, 1983). The gene has been isolated and cloned (Freytag, 1984), and citrullinemia is considered a promising candidate for early application of human gene therapy. Ornithine carbamoyl transferase deficiency can be quite severe, and the gene that codes for it has been cloned (Horwich, 1984), making it also a potential candidate for gene therapy. Lesch-Nyhan disease is a rare genetic disorder. It affects primarily boys who appear normal at birth but soon show abnormal uncontrollable movements. Abnormal behaviors of self-mutilation such as biting off fingers or otherwise inflicting painful injuries are part of the syndrome, as well as aggression towards others. These bizarre symptoms are extremely distressing to the patient and his family. Lesch-Nyhan syndrome is caused by complete deficiency of the enzyme

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hypoxanthine-guanine phosphoribosyl transferase (HPRT), the same enzyme that is partially deficient in gout (Wilson, 1984). The gene has been cloned (Miller, 1984; Jolly, 1982; Yang, 1984), and proposals for human experimentation on gene therapy for Lesch-Nyhan syndrome have been submitted to at least one local Institutional Review Board (Baskin, 1984; Merz, 1984). Proposals to begin human experiments on Lesch-Nyhan syndrome are expected to be referred soon to the National Institutes of Health (Anderson, 1984; Jenks, 1984; Merz, 1984).

It may be possible in the future to alter specific tissues while they are still in the body. It would be desirable, for example, to selectively alter nerve cells to treat diseases caused by metabolic disruption of brain cell function, or to correct only liver cells in genetic diseases that primarily affect proteins produced by the liver. The worst behavioral symptoms of Lesch-Nyhan syndrome, for example, presumably involve disruption of normal neural processes, and it might prove necessary to directly treat nerve cells. While methods for specifically targeting particular cells for directed gene therapy are theoretically possible, they have not yet been developed. Several possible methods of delivering specific genes to targeted cells may be found in the future, however, by use of tailored viruses or antibodies attached to artificial membrane sacs that contain the appropriate genes (see Technical Note 2).

Stages of development of gene *therapy technology*

If human gene therapy becomes a viable medical technology, its development will fall into several stages.

- Feasibility ***testing,*** involves animal studies and in vitro experiments on human cells, but not with patients.
- ***Early clinical research*** involves a few human patients with rare and severe diseases for whom other treatment alternatives are too risky, inapplicable, or less likely to be beneficial.
- Clinical ***testing*** will occur only if a potential for success has been demonstrated in early clinical research and feasibility testing. Clinical testing might involve a wider range of diseases and larger number of patients than early clinical research if experience with more severe diseases is fruitful. The final stage would be
- ***Standard medical practice*** in those specific instances where gene therapy has been shown safe and efficacious for a particular disease or type of patient. Issues of fair access to the technology, methods of paying for it, and proper quality assurance would emerge as the technology made the transition to this final stage.

Somatic cell therapy is now in the first stage, verging on the second. Germ line gene therapy has not even undergone feasibility testing in a form that might be applied to humans. Gene therapy for different disorders or specific kinds of patients will beat different stages of development; only a few diseases are now being tested for feasibility of somatic cell therapy (Working Group on Human Gene Therapy, 1984; Anderson, 1984).

Techniques of gene therapy

Gene therapy involves isolating a gene, putting it into cells where it will be used, and ensuring that the inserted gene functions in the new cells in a way that does not harm the patient.

Genes are copied and passed on by DNA replication

Genetic information is transmitted from one cell to its progeny by duplication, or replication, of its DNA. When a cell divides, it copies its DNA and distributes a copy to each of two offspring cells. A new therapeutic gene introduced into a cell in the laboratory can thus be reproduced through the process of cell division when the cell is placed into a patient and proliferates.

Many breakthroughs in molecular genetics have come from discoveries about how DNA replicates, how it can be specifically cut and reassembled, and how to re-introduce the altered DNA back into cells in such a way that its expression, or translation into protein, can be controlled (Judson, 1980). Many of the techniques for splicing and controlling the expression of genes were first discovered between 1970 and 1974, using some of the same techniques that led to the development of recombinant DNA (Watson, 1984).

Isolation and cloning of the normal gene

The usual first step in approaching gene therapy is identification of the abnormal gene. (This step can be skipped when the corresponding normal genes are already available, as was the case for sickle cell disease.) Once the abnormal gene has been found, then copies of the corresponding normal gene must be isolated and copied. There are several ways to identify abnormal genes. These involve analysis of patterns of inheritance of a disease, study of the metabolism of patients who have the disease, and analysis of the genes of those who have the disease, Identification of the gene that causes a particular disease requires hundreds of experiments, luck, and extensive resort to recombinant DNA technology.

Once the gene that causes a disease has been identified, the corresponding normal gene must be isolated, unless it is already available because it has been studied for some other purpose. Using an abnormal gene to find its normal counterpart is usually done by exploiting the extensive similarity between the sequences of the normal and defective genes; they rarely differ greatly in overall sequence (although the ***functional*** results are quite different, or there would be no disease).

After the normal gene has been identified and isolated, then it must be copied. The process of making multiple copies of a single gene is called cloning.[7] Cloning involves combining the gene of interest with DNA sequences that allow it to be copied in lower organisms—usually bacteria or yeasts. The DNA containing the gene of interest is then inserted into bacteria or yeast (or, more recently, into some types of mammalian cells growing in culture). The DNA is copied as the cells proliferate. The numerous copies of DNA are then purified from other cell components, and the gene of interest can be cut away from unwanted DNA sequences, One now has millions or billions of copies of a single gene.

These copies are then combined with DNA that is suitable for insertion into human cells.

Insertion into human cells

The DNA that contains the normal gene can be administered to human cells in several ways: using viruses, physically injecting it, treating the DNA chemically so that cells take it up, treating the cells so that they are induced to take in the DNA, or by fusing the cells with membranes that

[7] For details of cloning, see the Technical Notes. (honing a gene should not be confused with cloning an organism, The term "cloning" refers to reproduction without mating: in the case of a gene or DNA sequence, this merely means making copies of the relevant stretch of DNA. Cloning a whole organism, in contrast, involves copying ***all*** of a cell's DNA so that a completely new organism that shares **all its genes with the original is produced.** The **techniques tor** cloning genes are completely' different frum those for cloning organisms. Cloning an individual human would not help in the prevention of genetic disease, and is not directly related to the questions raised by human gene therapy

contain the DNA. In the distant future, designed viruses or genetic elements may be used to transfer genes to specifically targeted human cells. At present, however, more primitive methods are used.

VIRUSES

Viruses are small packages of genetic information in the form of DNA or RNA that enter cells and either insert their information into that of the infected cell or duplicate themselves using the cell's biochemical machinery. Viruses are usually covered with a coat of protein or membrane, but their most distinguishing characteristic is the genetic information that they contain. Some viruses promise to be practical for gene transfer because they are relatively simple and controllable, and contain sequences that permit insertion of genes into the host's DNA. Modified viruses are the most likely candidates for gene therapy in the long run, because they are highly efficient, can affect many cells, and are relatively easy to manipulate in the laboratory (Rawls, 1984).

Several scientists are developing viruses that would not injure cells, would not propagate uncontrollably, and would enter only target cells (Anderson, 1984). Such viruses have been successfully used to insert new genes into blood-forming cells of mice with relatively high efficiency (A. D. Miller, 1984; Williams, 1984). At some point, scientists may be able to design a virus that could be used for cloning as well as delivery, saving yet more steps.

MICROINJECTION

Microinjection of DNA involves putting the DNA one wants to insert into a solution that can be pushed directly into individual cells through extremely small needles made of glass. The technique is highly reliable, in that a high proportion of cells that receive genes express them (Capecchi, 1981), but limited by the number of cells that can be directly injected. Investigators can inject hundreds or thousands of cells, at most, for a given experiment, compared to billions that can be treated using viruses or chemical treatments. Microinjection has been the method of choice for experiments involving gene transfer in mice, because of its reliability, but its applicability to humans is questionable because it is not ***completely*** reliable, and often results in cell death (an alternative that is ethically unacceptable for human experiments) (Anderson, 1984).

CHEMICAL AND PHYSICAL METHODS

Some early experiments in gene transfer employed mixing DNA with chemicals and subsequently applying the DNA to a large number of cells. Most cells would pick up the DNA, and some would insert it into their own DNA, and, in some cases, express it. The usual chemical treatment employed calcium phosphate with relatively large amounts of the desired DNA. The most common physical method involved "electroporation") in which electrical treatment of the cells induced uptake of DNA and other constituents from the fluids bathing the cells.

Chemical and physical treatments have the advantage of not requiring a vector to cause insertion, but have two major disadvantages. First, the DNA is only stably incorporated into a small proportion of cells, usually only one in ten thousand to one in a million. (This small proportion nevertheless usually represents hundreds or thousands of times more cells than could be directly injected.) This feature requires that cells that take up and incorporate the desired DNA must somehow be separated from cells that do not, and there must be a very large number of cells to treat in the first place. Second, the DNA usually inserts at random into the cell's genome, and often in multiple copies. DATA insertion following chemical and physical insertion methods is thus relatively uncontrolled and unpredictable (Anderson, 1984).

MEMBRANE FUSION

The final way to get DNA into cells involves putting it inside of membranes that can then be fused with the outer membrane of target cells, allowing the contents to spill into the cells. The membrane sacs, called liposomes, can be made of artificially constructed lipid mixtures or derived from specially treated cells such as red blood cells or bacteria. The advantage of cell fusion is that it is relatively simple, and large numbers of cells can be treated. It is, like chemical treatment, unreliable and nonspecific at delivery. The technique might prove useful in the future, however, if membranes are constructed that target specific cells with highly reliable delivery.

Background on genetic diseases

Chromosomes and inheritance

Higher organisms package their DNA into segments called chromosomes. Each chromosome is composed of one very long stretch of DNA that is bound to various proteins and other molecules. There are two copies of each of 22 chromosomes in the cells of a human. In addition, there are two sex chromosomes. Females have two '(X" chromosomes and males have one "X" and one "Y. " In normal human cells, therefore, there are 46 chromosomes: 2 sex chromosomes and 2 copies of each of 22 other chromosomes (these non-sex chromosomes are called autosomes).

The 46 discrete aggregates of DNA and attached protein that comprise the chromosomes are maintained inside the nucleus of somatic cells. In germ cells, in contrast, a specialized phenomenon called meiosis takes place. Cells divide so as to leave only 23 chromosomes in a sperm cell or ovum: one sex chromosome (either an "X" or a "Y") and ***one*** copy of each of the autosomes. All ova contain an "X" and 22 autosomes, because they derive from female cells that contain two "X" chromosomes. Sperm are divided into two groups; half have an "X" and 22 autosomes and the other half have a "Y" plus 22 autosomes.

During the process of fertilization, a sperm joins with an ovum to restore the chromosome number to 46. If the sperm contains an "X" chromosome then a female is produced, and if it contains a "Y" then a male results.

Single gene, multigene, and environmentally modified traits

Diseases that might be treated by gene therapy will, at least in the foreseeable future, be exclusively those caused by mutations in a single gene. Such diseases are called single gene defects, and contrast with diseases and traits influenced by several genes or environmental factors. Genes can cause disease through several mechanisms. Most human diseases have a genetic component inherited by the individual and an environmental component that comes from outside the individual. The relative importance of genetic and environmental influences varies in both patients and diseases. Some medical conditions, such as automobile accidents or war wounds, may have large environmental and very small genetic contributions. Most diseases have a mixture of genetic and environmental contributions (Harsanyi, 1981). In several disorders, such as Huntington or Tay-Sachs diseases, the influence of a single gene is so large that the disorders are called genetic diseases.

SINGLE GENE TRAITS, OR MENDELIAN TRAITS

When traits or diseases are primarily determined by a single gene, they obey the relatively simple laws of inheritance first specified by Gregor Mendel, a monk who lived in the last century and whose interests in agriculture led him to discover several genetic phenomena in plants. The same patterns of inheritance that Mendel first described in plants, noted below, are also found in several human diseases, and thus indicate that the cause of the disease is genetic.

At the turn of the century, a British physician and scientist, Archibald Garrod, first introduced the idea that some diseases that followed definite inheritance patterns might be caused by "inborn errors of metabolism" (Stanbury, 1983; **Kevles,** 1984). He postulated that some diseases were due to biochemical errors. He further speculated that such biochemical defects might be caused by genetic abnormalities that obey Mendel's laws. Several decades later, biochemical errors were, for first time, traced to specific enzymes. These discoveries confirmed Garrod's hypothesis. Other diseases were traced to molecular defects in non-enzyme proteins; the first "molecular disease" described was sickle cell anemia, in which an abnormal hemoglobin protein was found (Pauling, 1949). Research over the past three decades has revealed more and more genetic diseases, and greater understanding of many of them.

Many genetic diseases are due to changes in just a single gene, such as ADA and PNP deficiencies. More than two hundred specific enzyme defects

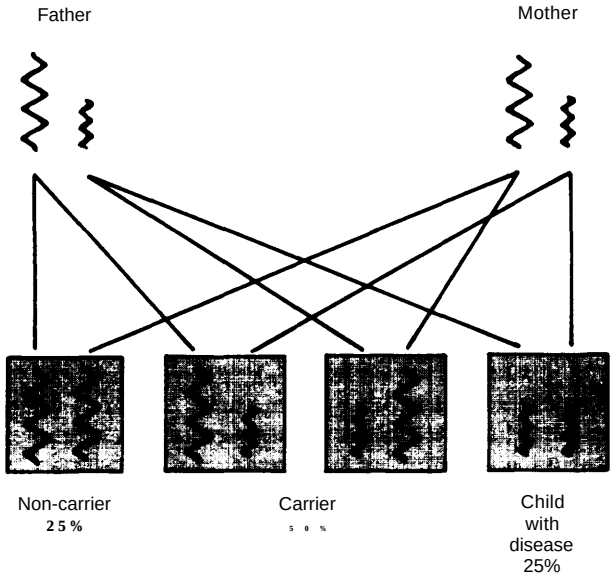
cause known human clinical syndromes, and over a hundred other genetic diseases have been biochemically characterized (Stanbury, 1983). Prominent disorders such as sickle cell anemia, familial hypercholesterolemia, polycystic kidney disease, Huntington disease, neurofibromatosis, Duchenne muscular dystrophy, cystic fibrosis, achondroplasia, hemophilia, and many others are examples of single gene disorders. Many common adult disorders, usually excluded from pediatric statistics on genetic disease prevalence, such as Alzheimer disease and hemochromatosis, have forms strongly influenced by genetics (Breitner, 1984; Cook, 1979, 1981; Folstein, 1981; Cartwright, 1978, Dadone, 1982; Skolnick, 1982; Kravitz, 1979). Single gene defects affect 1 to 2 percent of newborns (Lubs, 1977), and addition of adult genetic diseases would significantly increase the estimated prevalence and cost of genetic disease.

Even diseases or traits that are due to a single gene vary widely in severity, depending on environmental factors and other genes; the extent to which patients have signs and symptoms of a genetic disease is called "expressivity." Diseases can also be variably expressed in populations, affecting some people and not others who carry the gene. This is described as "penetrance." Complete penetrance indicates that all who have the defective gene also have the disease, while incomplete penetrance means that some people have the gene but not the disease.

Single gene traits can be classified by how they are inherited. They can be recessive, dominant, or X-linked.

Recessive Disorders.--Recessive diseases occur when one receives a defective gene from both parents (see diagram). Diseases due to dysfunctional gene pairs are usually due to protein abnormalities that cause a biochemical imbalance. Sickle cell disease and thalassemia affect globin, the protein part of hemoglobin, which transports oxygen through the blood to body tissues. Other recessive disorders, such as Tay-Sachs disease, ADA and PNP deficiencies, and phenylketonuria (PKU), affect enzymes whose absence or dysfunction adversely affects cellular metabolism. Most of the relatively well understood genetic diseases are recessive disorders that can be traced to specific defects in enzymes. However, the specific molecular defect underlying many recessive diseases, including at least one common one-cystic fibrosis—is not known,

Recessive **Inheritance**

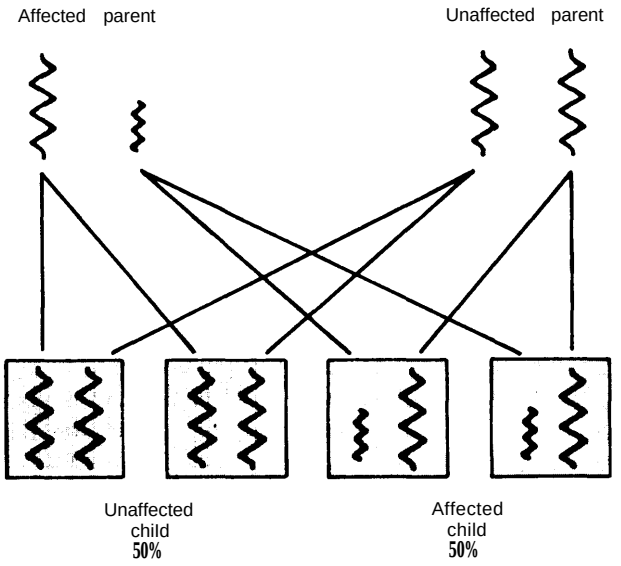


SOURCE: Office of Technology Assessment.

Dominant Disorders.--Dominant disorders occur when offspring receive a defective gene from either parent, and having just one such gene leads to expression of the disease (see diagram). In some cases the defect is known, such as some types of porphyria, in which enzyme deficiencies lead to abnormal disruption of biochemical pathways that produce and degrade heme—the non-protein part of hemoglobin found in red blood cells. In most dominant disorders, however, the biochemical nature of the derangement is not known; the molecular defect in dominant disorders is, in general, less well established than for recessive ones.

X= Linked Disorders.—X-linked disorders are carried on the "X" chromosome. X-linked diseases usually affect boys because males have only one copy of the "X" chromosome: there is no set of genes on a second "X" chromosome to balance the effects of a defective copy of the gene. The inheritance pattern of X-linked disorders is distinc-

Dominant Inheritance



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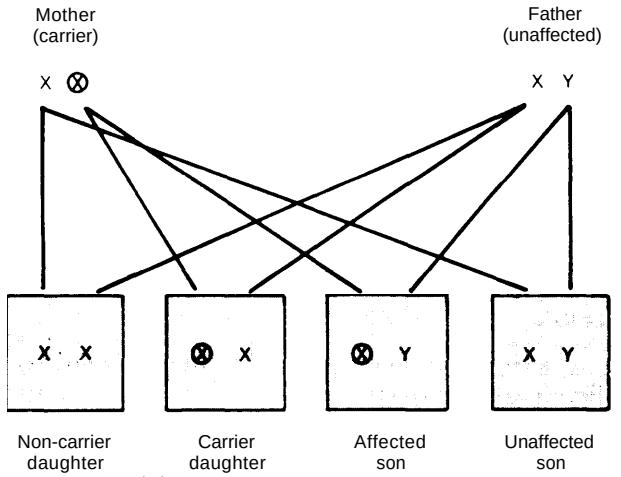
X-Linked Inheritance



SOURCE" Off Ice of Technology Assessment

tive: sons inherit the traits only from their mothers, because a son always derives his "X" from his mother and his "Y" from his father. Daughters can get a defective gene from either parent, but do not usually have the disease unless they get the abnormal gene from ***both*** parents. X-linked traits thus act like dominant traits inherited only from the mother in boys, and are usually recessive in girls. Examples of X-linked disease traits are hemophilia, Duchenne muscular dystrophy, and Lesch-Nyhan syndrome.

There are apparently few traits, and no known diseases, that are carried in genes located on the "Y" chromosome, and expressed only in males.

Single gene defects are, in general, the best understood of genetic diseases; the early instances of human gene therapy will be done to correct the effects of single mutant genes.

MULTIGENE TRAITS

There are certain body characteristics and other traits that accrue from the interactions of several genes. Eye and hair color, for example, are traits that are specified by genes, but do not obey simple Mendelian patterns of inheritance because many genes are involved. Similarly, there are genetic diseases caused by interactions of multiple genes that are minimally affected by environmental influences. Such disorders are termed polygenic or multigenic.

ENVIRONMENTALLY MODIFIED TRAITS

The vast majority of characteristics that define individuals are determined by a combination of genetic predisposition and interaction with the environment. Height, for example, though determined genetically to a significant extent, is also influenced by nutrition and other factors. Likewise, many diseases derive from interactions of genes and the environment in which both components contribute significantly to the disease process.

The type of diabetes mellitus that occurs in younger people, for example, is now believed to be caused by a special susceptibility of insulin-secreting cells to certain viral infections or other environmental insults. The clinical disorder is thus caused by an environmental agent acting in concert with genetic characteristics. Most common diseases, including cardiovascular diseases, cancer, and many drug reactions appear to involve multiple genes as well as environmental influences.

Most complex human traits, including physical and intellectual capacities, are also multigenic and

environmentally influenced. The controversies that have raged in the psychological literature over the genetic and racial components of intelligence center on the relative importance of genetic and environmental contributions, including nutrition, health care, cultural background, and socioeconomic status. There is little doubt that genetics and environment interact, but there is vigorous contention about which factor predominates and how public policy should respond to differences in complex traits such as intelligence,

Genetic treatment versus eugenics

Eugenics is the term applied to "the 'science' of improving human stock by giving 'the more suitable races or strains of blood a better chance of prevailing speedily over the less suitable' " (Kevles, 1984, quoting Francis Galton). The eugenics movement is noted for promulgating social programs intended to enhance desired human traits, such as intelligence and physical strength, and to eliminate undesirable traits, such as "feeblemindedness)" criminality, and disease.

Eugenic social movements date back to the last century, and the intellectual history of eugenics extends much further back in history (Kevles, 1984). Deliberate eugenic interventions have been decried several times in the 20th century. Eugenic movements were popular throughout Europe and the United States early in this century, and were most overtly expressed by the National Socialist (Nazi) Party in Germany before and during World War II.

Federal legislation to restrict immigration passed between World Wars I and II was based, in part, on eugenic principles, and mandatory sterilization laws also supported by eugenicists still exist in several States (Kevles, **1984;** Reilly, 1977) and are occasionally used (Bowman, 1984). Many prominent geneticists were involved in the American eugenics movement in the early part of this century (Kevles, 1984), and participated as "experts" in preparing legislation or otherwise promulgating social reform congruent with eugenic aspirations.

Germs of eugenic thought persist in contemporary society, notably in regard to controversies about genetics and intelligence (Lewontin, 1984), and some fear that the technical advances of molecular genetics may lend themselves to abuse (Grobstein, 1984; Reilly, 1977). This is not a criticism of the technology per se, but rather a concern about its potential misapplication or affiliation with forms of social coercion (Powledge, 1984).

The distinction between gene therapy and eugenics rests on several different points. Gene therapy involves the informed participation of patients who suffer from a specific disease, while eugenics involves social programs, sometimes involuntary ones, focused on general human traits. Gene therapy is intended to benefit a particular individual, while eugenics is intended to improve the human general (or, often, national) population. Gene therapy is directed at correction of symptoms due to genes known to cause disease, while eugenics often dwells on polygenic traits whose genetic components are controversial, and whose expression is poorly understood.

Medical, scientific, technical, ethical, religious, and social commentators have noted the difference between therapy for a specific genetic disease and interventions intended to enhance traits such as intelligence and physical appearance (Siegel, 1982,1983; Fletcher, 1983b; Friedmann, 1983). Genetic correction of specific diseases, if it does not affect the germ line, is analogous to other medical procedures that involve risk assessment by the patient and attending health professionals.

There is not a complete dichotomy, however, between the correction of specific diseases and eugenics based on social preferences for certain traits. This is relevant in considering gene therapy because it vitiates any sharp distinction between correction of a specific genetic defect, which might be treated by gene therapy, and affecting a mildly undesirable trait, for which gene therapy might be controversial. A genetic condition might be considered serious by one person, and not by someone else. Baldness or brown eyes, for example, might be considered treatable

genetic defects by one family, and would scarcely be noticed by another.

The distinction between individual decisions in favor of gene therapy and social programs advocated by eugenicists can also blur if gene therapy becomes commonplace. Many individual decisions can culminate in wide social effects. The social impact of gene therapy depends on how often it is used, who has access to it, which conditions are treated, and what public policies are erected to foster or inhibit it. As long as gene therapy is restricted to rare recessive disorders, it will likely have minimal social risk and large benefits to individual patients.

Application of gene therapy to enhance traits such as intelligence or physical strength cannot now be done because so little is known about the genetic influence on such traits. Most traits that some individuals might consider desirable to amplify will likely prove to be polygenic or environmentally influenced, and thus technically approachable by gene therapy only in the distant future, if ever. There is no guarantee, however, that it will always be impossible to use the techniques developed for gene therapy to improve socially esteemed mental or physical traits in at least some patients. If desirable traits can be modified by methods developed for gene therapy, then public policy for such applications may well prove analogous to those now employed for cosmetic surgery. Cosmetic surgery is not generally reimbursed as part of government or private health insurance, but is usually paid directly by individuals. Cosmetic surgery has not generated major public policy dilemmas, although controversy might arise in gene therapy if parents were attempting to secure "cosmetic" gene therapy on behalf of an unborn infant or young child, or to authorize germ line changes that would not be reversible in future generations.

Medical aspects of gene therapy

Early clinical experiments in human gene therapy will be performed on somatic cells of patients to attempt partial correction of life-threatening diseases. They will be performed to allay the signs and symptoms caused by a defect in a single gene whose normal counterpart has been cloned, and whose correction does not require careful control of expression. Gene therapy will be considered when there is no preferable alternative treatment available to the individual patient. This prediction is based on analysis of several factors described below, and underlies the analysis throughout this section. Predictions about human gene therapy are based, in part, on results of animal experiments. A short review of such animal experiments is followed by a discussion of relevant clinical considerations in humans. The medical aspects of gene therapy include reasons that genetic diseases can never be completely eliminated from the population, why certain types of genetic diseases are not good candidates for gene therapy, why germ line therapy may never be necessary or its use extremely restricted, and which disorders might be approached using gene therapy in the near future. The analysis is restricted to the early applications of human gene therapy because technical predictions beyond this time horizon are perilous, and because decisions confronting Federal policymakers in the next few years will be focused on early applications.

Genetic corrections of animals and other organisms

Gene therapy is contemplated in humans only because it has been performed in animals and lower organisms. One of the most successful attempts to genetically alter organisms involved the "cure" of a genetic defect in fruit flies (Spradling, 1983). Some fruit flies have an enzyme defect that results in their having rose colored eyes. Scientists were able to correct this abnormality by delivering the correct gene into fly cells by using DNA molecules specific to fruit flies that can carry foreign DNA into the fly's own DNA. The treated flies that took up the normal gene transmitted the

genes to their progeny, who showed normal eye color.

Gene transfer experiments have also been done in mice. Several traits have been artificially added to mouse cells early in embryonic development. In experiments involving transfer of rat growth hormone to mice, the mice that develop from the altered embryos express the foreign genes, although not in a way that is controlled like the normal gene would be. Scientists have had to use special techniques to get mammalian cells to incorporate new genes, and the genes are inserted into chromosomal or cellular locations that cannot be predicted or controlled. Examples of genes that have been transmitted to progeny in mice include the gene for rabbit hemoglobin, rat growth hormone, and a DNA fragment with both specific enzyme activity and antibiotic resistance to neomycin (Palmiter, et al., 1982; Palmiter, et al., 1983; Brinster, 1983; Wagner, et al., 1981; Williams, et al., 1984).

The growth hormone experiment was especially interesting because expression of the gene could be manipulated by the scientists, and this feature was inherited by progeny of the treated mice. Other transferred genes have also been passed to the progeny, although genetic manipulations have occasionally resulted in undesired side effects, such as sterility or induction of new mutations. These effects suggest that oversight committees will seek evidence that such side effects are highly improbable when inspecting proposals for experiments that involve human gene therapy (Working Group on Human Gene Therapy, 1984).

Most of the animal experiments noted above resulted in germ line changes of the treated mouse lines, The experiments were done to investigate animal development, rather than to pave the way for human application of gene therapy. More recent experiments have been done on somatic cells of animals, and are more directly analogous to what would be done in early human trials. Several groups of investigators have successfully inserted genes into the bone marrow cells of mice, and have shown production of proteins from the inserted genes in cells that derive from bone marrow cells (Kolata, 1984c; **A.** D. Miller, 1984; Williams, 1984; Anderson, 1984). These experiments used modified viruses as the gene transfer agents in ways quite similar to those that might be used in humans, although treatment of the recipient mice was more drastic than may be acceptable for humans, and data on long-term risks (e. g., reversion to infectious virus type, induction of new mutations, predisposition to cancer, and integration into the germ line) were not reported. The new studies show great promise, and demonstration of technical feasibility should encourage animal experiments to ascertain the magnitude of the risks.

Other recent experiments demonstrate that proper regulation of gene expression in the cells of humans and other higher animals is more complex than in fruit flies and bacteria. Early attempts at gene therapy in humans will probably, therefore, be conducted on diseases for which there is reason to believe that precise regulation is unnecessary for therapeutic benefit, such as ADA and PNP deficiencies. Early plans to apply gene therapy to diseases in which regulation would be important have been thwarted by the complexity of regulation, although such obstacles may eventually be overcome. Hemoglobin disorders, for example, will not be the first candidates for human gene therapy because of the need for regulation of globin expression (Anderson, 1984).

Reasons genetic diseases cannot be eliminated

There will always be patients who suffer from genetic diseases. It will never be possible to eliminate even single gene defects, although the prevalence of some disorders, especially some dominant ones, could be significantly reduced. New mutations causing genetic defects will always occur, and so people will be born carrying such mutations. Neither would it be possible to stop the expression of recessive diseases by preventing those who carry one copy of any abnormal gene from mating, because humans carry an estimated 5 to 10 recessive defects in their genome on average, and so no one would be permitted to mate.

It is already possible to prevent the birth of children with some genetic disorders through

genetic counseling, prenatal diagnosis, and family planning. The number of diseases for which this is possible will grow as we learn more about human genetics. It is unlikely, however, that current methods for preventing genetic disease will prove practical for all, or even a large fraction of couples in the near future. Most genetic disorders still cannot be detected prenatally, and tests for carriers are available for even fewer diseases. Yet effective prevention requires such tests. Furthermore, such tests must not only be available; they must also be used. Barriers to use include cost, complexity, and lack of public awareness. Given the large number of potential genetic diseases, it is unlikely that any one screening test will screen for all, or even most genetic diseases. This means that for many disorders, couples will only know that they are at risk after an affected child has already been born. Thus, until the entire child-bearing population is screened for a given defect, or prospective parents know of special risks, even those diseases for which all the relevant tests are available will persist.

It maybe useful to screen some populations for some defects. Screening programs for Tay-Sachs carriers among Jews of Eastern European descent, and for thalassemia among Mediterranean populations have been successful in some instances. These successes cannot be generalized to all genetic diseases, however, and are probably relevant only to a few relatively common disorders.

Effective use of genetic screening and selective testing presumes public awareness that such tests are available and acceptable. Families must wish to use the technologies and expect to benefit from the information provided. This requires that there be no stigma attached to carrying a potential genetic defect and trust that genetic patient data will be properly used. (Issues relating to control of and proper access to genetic patient data are discussed below, and in app. B.)

There are a few genetic diseases whose prevalence could be dramatically reduced. Huntington disease is a dominant trait encoded in chromosome 4 that causes a debilitating brain disease that usually becomes evident only in a patient's 40s or 50s (after reproductive decisions have been made). All those who carry the gene for Huntington disease will develop the disease if they live long enough. If carriers could be told whether or not they had the gene before deciding to have children, and if all those who carry the gene decided not to have children, then the gene could be eliminated in a single generation. This is true of Huntington disease because it is almost always inherited and only rarely due to a new mutation (this is not true for many dominant disorders).

Elimination of the gene would, however, entail large numbers of coordinated personal decisions. Marjorie Guthrie, wife of the famous folk singer Woody Guthrie who was afflicted with Huntington disease, posed a difficult question that bears on any program to prevent the birth of those with Huntington disease, "Does anyone really think it would have been better for Woody not to have come into the world-in spite of everything?" (Cited in Rosenfeld, 1984). Is the disease so awful that the birth of potential Huntington patients should be prevented when they would have several decades of relatively normal life? This is just one of several difficult dilemmas that emerge from advances in genetics related to particular diseases. New genetic technologies for determining the genetic makeup of humans may provide the information about whether one is susceptible to Huntington disease[8] and other disorders, but cannot determine a moral choice that involves social, religious, and personal values. In the absence of compelling social justifications, decisions are and should be left to individuals, families, and health professionals in a particular situation.

Even if all diagnostic tests are available, there are families for whom the prospect of selective abortion is unacceptable, or who choose not to avail themselves of genetic testing technologies for other ethical, religious, legal, social, or medical reasons. Such couples, while not at increased risk of having children with genetic diseases, will nevertheless inevitably bear some children with genetic defects. The only way to avoid this would

[8]A method of detecting Huntington disease before symptoms emerge, and even before birth may he available within a decade—a technique is already available for certain families (see app A; Wexler, 1984; Gusella, 1%43: Rosenfeld, 198.$).

be to circumscribe their liberty, making the judgment that the potential social benefit overrides their autonomous right to choose what is best for themselves and their families. The generally high regard for personal autonomy in our society implies that such couples' right to make reproductive decisions will be protected.[9]

Existence of new mutations, absence or unavailability of genetics tests, and freedom of choice all suggest that genetic diseases will continue to exist, and therapies for them in infants, children, and adults will continue to be needed.

Types of Genetic Disease That Are Poor Candidates for Gene Therapy Now

CHROMOSOMAL DISORDERS

In addition to genetic diseases that are caused by mutation of single genes or small numbers of genes, there are others caused by abnormal chromosomes. One group of genetic disorders is defined by a surfeit or deficit of chromosomes in cells of the affected individual: patients have an abnormal number of chromosomes or parts of chromosomes. The most common such disorder is Down syndrome, which affects one in 600 live-born infants. Chromosomal disorders overall affect one in 200 newborns, and account for half of all spontaneous abortions (Burrow and Ferris, 1982).

Gene therapy for chromosomal disorders is not scientifically possible now, even in experimental animals. Chromosomal abnormalities involve the improper placement, absence, or duplication of fragments of chromosomes or entire chromosomes. Chromosomes typically contain hundreds or thousands of genes, and there are no techniques presently available for inserting enough DNA to correct such large defects in either somatic or germ cells.

COMPLEX AND DOMINANT TRAITS

At present, there is a large technological gap between those diseases for which gene therapy is promising in the near term and those about which so little is known that gene therapy cannot even be rationally contemplated,

Complex traits such as intelligence and physical stamina, are not sufficiently understood to merit serious contemplation of any genetic intervention, and gene therapy could certainly not be justified, both because such intervention might not be considered '(therapy, " and because there is no gene whose insertion would likely be effective. Even if gene insertion could reliably alter physical and mental abilities, many question whether it would ever be used, because it would have to be cheaper and more effective than other techniques for altering human characteristics. Genetic techniques would have to prove more effective or less costly than education, indoctrination, physical and mental training, and drugs.

Dominant traits, and ***poorly understood recessive diseases*** are also poor candidates for gene therapy in the near future. Therapy of such disorders will depend on the specific cause and biochemical or metabolic manifestations of the disorder. To date, no dominant disorder is sufficiently well understood to warrant an attempt at gene therapy.[10] **There are,** however, a few dominant traits that could potentially be treated using gene therapy. Gene therapy might eventually be contemplated for those enzyme defects inherited as dominant traits, and for diseases caused by deletions of small amounts of DNA that could be replaced (there is some evidence for such deletions in retinoblastoma and Wilm's tumor—cancers usually developed in childhood that are inherited as dominant traits). In such cases, the decision to undertake somatic cell gene therapy for the dominant disorder will not significantly differ from consideration of recessive traits. Nevertheless, few dominant disorders have been characterized biochemically, and simple gene insertion may not correct many dominant disorders. Correction of dominant diseases may require insertion of extensive amounts of DNA, gene surgery to remove the defective gene, or both; techniques for these more complex manipulations have not been demonstrated in mammals. Prospects for gene therapy of dominant disorders are therefore, in general, poorer than for recessive enzyme defects, although a few dominant diseases might be addressed.

[9]L. Andrews, 1984d, citing ***Carey*** v. ***Population Services International,*** 431 U.S. 678, 685 (1977).

[10]This generalization does not apply to traits that are dominant in males and recessive in females (X-linked traits).

Reasons Germ Line Therapy May Be Unnecessary

Germ line gene therapy may never be widely practiced because treatment of abnormal embryos and gametes offers little advantage over selection of normal ones.

Germ line therapy, as currently practiced in animals, involves taking embryos in vitro, genetically altering them, and returning them to a female for further development. In early embryonic stages, only a few cells are present. To determine whether the embryo is normal or abnormal would require that one have a test that provided a diagnosis without disrupting the few cells. No such tests exist at present.[11] There are prenatal diagnostic tests, but these are useful only later in pregnancy, when many more cells can be sampled to make a diagnosis without harming the fetus, 12

In order to practice gene therapy on an early embryo, one would have to treat either all embryos or only ones known to have a treatable genetic defect (Harsanyi, 1982; Pembrey, 1984). Treatment of just those embryos carrying genes for a particular disorder would require a way to identify them. If methods to identify embryos carrying the abnormal gene were available, though, it would be easier and safer to merely select a normal embryo rather than treat an abnormal one (Harsanyi, 1982). If all embryos are treated, then a significant fraction of normal embryos would be unnecessarily subjected to the added risks of gene therapy manipulations, The ratio of normal to abnormal embryos depends on the type of genetic defect being treated. In the most common scenario, involving two parents who are known carriers of a recessive gene, only one in 4 embryos would develop the disease, and so one unaffected and two asymptomatic carrier embryos would be treated for every one in which the disease was prevented. If parents have dominant or X-linked traits, at most half those treated would develop the disease. The situations described are those that would yield the highest fractions of abnormal embryos; most other types of traits would have even less favorable ratios of affected to normal embryos.

Gene therapy on embryos is also made less likely because of the need to ensure that it has been successful. Unless gene therapy were almost certain to work, parents might seek to determine that the defect had been corrected, much as they can now ask for prenatal diagnosis. Checking the success of gene therapy would require either a test for the embryo before it were reimplanted, none of which exists, or availability of a test later in pregnancy and before delivery. If such a test were available, it could be used for conventional prenatal diagnosis. Gene therapy of embryos would thus not avoid the ethical dilemmas already associated with conventional prenatal diagnosis, and would offer little advantage over selection of normal embryos or fetuses, while significantly increasing risks. For cases in which parents did not wish to check on the success of gene therapy, because of religious convictions or because they would not change their actions based on prenatal tests, this argument would not apply.

There are certain situations in which germ line gene therapy might be contemplated. For example, if a man and a woman both had PKU or sickle cell disease and wished to have their own children, then the parents and physician would know in advance that all embryos would acquire the disease because of the parents' genetic constitution. This situation would eliminate the risk of unnecessarily treating unaffected embryos, but might still require a method for ensuring that the gene therapy had been successful (although parents might choose not to test this because of personal or religious beliefs).

The strength of the arguments against germ line gene therapy would also diminish if gene transfer techniques became extremely reliable. How-

[11] Techniques for separating animal embryos and growing identical twins from them have, however, been developed (Maranto, 1984b). These same techniqes, if applicable to humans, might eventually be used to do diagnostic tests on cells separated from the embryo early in development. This would permit preimplantation and later prenatal genetic screening, and might also allow monitoring of the efficacy of gene therapy without harming the embryo or fetus. This might, however, be ethically unacceptable.

[12] There are economic and technical reasons, however, to intensify the search for techniques to detect genetic defects in single or small groups of cells in early embryonic development. Techniques of *invitro* fertilization involve great economic cost and failures cause severe emotional distress; in this setting, a premium is placed on ensuring the normal status of embryos before they are implanted.

ever, this would require dramatic technical improvements in gene transfer and would not eliminate the ethical dilemmas.

The medical complications of gene therapy suggest that germ line therapy on early embryos may never be ethically acceptable, even if it becomes technically feasible, except in extremely rare matings between parents whose genotype for a genetic disease is known. Uncertainty about possible effects of such therapy in future generations may preclude application of germ line gene therapy for even these instances.

Criteria for Beginning Human Gene Therapy

The decision to approve the application of gene therapy to humans should depend on satisfaction of several requirements. The requirements will be based on analysis of risks and benefits for the individual patient and consideration of the wider implications of approving gene therapy for any given patient. The factors considered in analyzing which applications of human gene therapy might be approved will include potential effectiveness, safety, reliability, presence or absence of alternative treatments, severity of symptoms, and prognosis. Each of these will be considered in relation to a particular genetic disease in an individual patient. Some generalizations about these factors, however, apply to the technique of gene therapy as a whole.

SAFETY

Judgments of the safety of gene therapy will be based on animal data and comparison to similar human interventions. For those few genetic disorders, such as thalassemia, that have counterparts in animals, short term safety can be assessed by experiments that measure clinical improvements in animals. For other diseases, it will be necessary to base judgments of safety on animal data obtained in experiments that involve gene transfer, although clinical benefit in the animals cannot be measured. Experiments might be performed, for example, using the same gene and delivery system as would be used in humans, and the animals observed to see if they express the gene or develop side effects.

Questions of safety include not only short term effects, but also long term consequences that may require years to ascertain even in animals (if such long-term risks can be assessed at all). Intergenerational effects would be especially difficult to assess, but would be of concern only if germ line cells were affected. Long-term studies of multiple generations of animals may also be required when and if germ line therapy is ever anticipated.

Defects that could affect a patient's progeny would be a concern if germ cells were affected by gene therapy. Protocols for human gene therapy of somatic cells will therefore be reviewed for evidence that ensures that germ cells are not affected (Working Group on Human Gene Therapy, 1984). The risk of germ line effects has precedent in cancer chemotherapy, radiation therapy, and some types of vaccination. Each of these technologies has a risk of inducing new mutations in the patient that could be passed onto the patient's progeny. If somatic cell gene therapy is done outside of the body, the risk of germ line effects is likely to be extremely remote. If, however, experiments involve administration of gene therapy to the whole patient, then germ line side effects will be a concern, and such risks must be outweighed by the severity of the disease or the magnitude of potential benefit in the individual patient. In the case of ADA or PNP deficiency, for example, the length of the patient's life would be less than 2 years and would be of low quality without gene therapy. For such a patient, the risk of germ line effects might be acceptable, particularly if such effects could be detected and the patient's reproductive decisions informed by this knowledge.

There are some special risks of using viruses to transfer DNA, and assurances of the safety of such transfer viruses will be prominent in approval of human experiments (Working Group on Human Gene Therapy, 1984). The special risks of viruses include the possibility of rearrangement of genetic material in the host that would lead to formation of an infectious agent. It is quite probable that scientists will be able to design DNA

derived from viruses that cannot revert to its more infectious form (Rawls, 1984; Anderson, 1984).

One special concern relates to the potential mutagenicity and carcinogenicity of gene therapy using techniques now available (Rawls, 1984; Anderson, 1984). It is not yet possible to control how and where inserted DNA integrates into that of the host cell. Insertion of genetic material may thus lead to new genetic mutations in the cells so treated (Gordon, 1981). It has also raised the prospect that inopportune insertion of new DNA may rarely cause or predispose a patient to develop cancer. Recent evidence about cancer genes suggests that certain cancers may be associated with abnormal expression of genes that are present in normal cells. Abnormal expression has been induced by viruses similar to those that are being developed to facilitate gene transfer, and cancer-like characteristics have been induced by techniques that closely parallel other methods that might be used for gene therapy (Hayward, 1981). The frequency with which gene transfer results in deleterious mutation or predisposition to cancer appears quite low, perhaps one in ten thousand to one in a million, suggesting that risks may well be less than for cancer therapy, immune suppression, or radiation (Working Group on Human Gene Therapy, 1984). Nevertheless, evidence for low risk of carcinogenesis will be explicitly sought in the approval process preceding early clinical trials (Working Group on Human Gene Therapy, 1984).

The short- and long-term risks of gene therapy are not known. It is thus inappropriate to attempt gene therapy except in the face of otherwise extremely poor prognosis until more is known about the risks. Determination of safety will likely derive from observations of animal experiments and the early instances of human gene therapy undertaken in patients with severe diseases—such as ADA deficiency, PNP deficiency, urea cycle defects, or Lesch-Nyhan syndrome–that lack a preferable alternative therapy in a given patient; for such patients, even a low probability of benefit may outweigh the uncertainties and risks of treatment. If animal experiments and early human applications prove safe, diseases with somewhat better prognoses might then be treated by gene therapy.

EFFICACY

Human gene therapy should not be approved until there is evidence that it might work; codes of research ethics require this. Commencement of experimental human gene therapy will require evidence from tissue culture and animal experiments. in the small number of diseases for which there is an animal model, judgments of efficacy can be based directly on clinical correction of animal diseases. In other diseases, constituting the majority of genetic disorders, it will be necessary to base judgments on studies in tissue culture, related human diseases, and relevant animal studies. Experiments might produce evidence, for example, that the human gene were expressed in treated animals or could be expressed in the patients' cells in vitro. The disorders in which gene therapy might soon be attempted do not have exact animal models, and so the earliest experimental human treatments may well be based on tissue culture studies and indirect animal experiments.

Demonstration of efficacy will require evidence that a gene can be delivered to a tissue where it can be effective, that it will remain in cells long enough to have an effect, and that the product of the gene is sufficiently expressed. In some future cases, these factors may require that the transferred gene serve as a direct replacement for the abnormal host gene, occupying the same location in the same tissue. In other cases, including those for which gene therapy is being seriously considered now, it may not be necessary to correct the defect so precisely.

In the case of ADA or PNP deficiency, for example, it may require only a little enzyme produced in bone marrow cells to sufficiently compensate for the biochemical defect. The absence of animal models indicates that the only way to test this is to do a human experiment. This is seriously considered for ADA or PNP deficiencies only because the diseases are rapidly fatal and there is, for most patients, no alternative therapy. Evidence for potential patient benefit for these

diseases may thus require only that the ADA or PNP enzyme be detected in bone marrow cells of the patient following gene transfer.

Genetic diseases that affect the brain constitute a particularly large group of disorders for which the question of organ specificity is crucial. There are several dozen genetic diseases whose most prominent symptoms are neurological, including Tay-Sachs disease, metachromatic leukodystrophy Lesch-Nyhan disease, and phenylketonuria (PKU). The brain differs from other organs in two important respects. First, the nerve cells, whose impaired function gives rise to symptoms, do not proliferate like bone marrow cells after they mature. This implies that genetic material introduced into one nerve cell cannot be amplified by allowing that cell to reproduce for many generations. Second, the brain has highly selective mechanisms for transporting substances from the bloodstream to brain tissues. Correction of biochemical defects elsewhere in the body may therefore not correct the defect in the brain, and may not eliminate neurological or behavioral symptoms.

Doctors and scientists do not know which brain defects can be corrected only in brain cells and which might be treated by modifying other tissues. Lesch-Nyhan disease is due to the absence of HPRT enzyme in all cells. Its worst symptoms are due to disruption of brain functions. There is uncertainty about whether or not the disease can be treated by correcting the biochemical abnormality in cells other than brain cells (e.g., bone marrow cells) (Anderson, 1984; Merz, 1984). Further, there is no way to test whether treatment of bone marrow cells would cure the brain dysfunction except through human experiments. If the disease could be treated by alteration of bone marrow, then patients who already have this severely debilitating disease could be treated. Otherwise, the only currently conceivable alternatives are treatment of cells early in development (that might also entail germ line changes), or prevention of the disorder by prenatal diagnosis and selective termination of pregnancy.[13]

[13]Other alternatives, such as implantation of genetically altered nerve cells or insertion of genetic material using engineered viruses specific for nerve cells, are theoretically possible, but have never been successfully demonstrated, even in animals.

Many questions about efficacy will be addressed by future genetic and clinical research. Determinations about which diseases can be treated and which methods are most successful must be made before human gene therapy becomes routine medical practice.

RELIABILITY

Experimental or medical therapy should be undertaken only if the procedures are sufficiently reliable to suggest that the potential scientific and clinical benefits outweigh the risks of ill effects or failure.

Animal experiments involving gene transfer, with the exception of those done in lower organisms, until recently had a relatively low probability of success in any one organism. This was tolerable to the investigators because their interest was in gene expression and animal development, and they could select the most scientifically interesting result from a large population of therapeutic failures. Such techniques are ***not*** acceptable for correction of genetic diseases in humans, where there must be of potential benefit to the individual treated.

Application of gene therapy in humans is now seriously considered only because of advances in the methods of delivering genes into cells and stable expression of genes so delivered (Anderson, 1984).

ALTERNATIVE TREATMENTS

Gene therapy will be acceptable only if it offers the best prospect of success among all potential treatments for a given patient. Factors that might be considered in comparing gene therapy to alternatives will include educated judgments about:

expected efficacy,
anticipated costs (to the patient or overall), and
magnitude and type of risks.

Such judgments will vary from physician to physician and patient to patient, as for any medical technology.

The genetic basis of a disorder does not imply that its treatment must also be genetic. There are several treatments that have proven effective in

some genetic diseases. The clinical manifestations of hemochromatosis can be prevented by periodic blood donation. Dietary treatments of PKU, galactosemia, urea cycle defects, and several other disorders considerably improve patient prognosis, although they are only partially effective and impose substantial limitations on patients and their families. Vitamin supplementation of those with Wernicke-Korsakoff encephalopathy and several other disorders can be quite effective.

Drug treatments can compensate for some genetic defects. Clinical investigators have already discovered two drugs that lead to partial correction of sickle cell disease by inducing expression of a type of hemoglobin, normally only expressed during fetal development, that can compensate for the errant sickle cell protein (see Technical Note 4). Clotting factors can be given to hemophiliac patients, and biotechnology may greatly increase the availability and reduce the cost of such factors.

Clinicians have also pursued the possibility of directly administering enzymes that are missing due to genetic defects (Desnick, 1981). Such enzyme therapy has not been clinically successful, but advances in drug administration could render such therapy practical. Development of drug pumps that reside in the body and deliver hormones, enzymes, or other chemicals for long periods of time may reduce the need for gene therapy. A new insulin pump developed by NASA, for example, promises to work for years without need for battery replacement (Langone, 1984).

Gene therapy is not the only way to restore normal genetic information to some organs of a patient with a genetic disease; some genetic defects may be remedied by transplantation of whole organs or tissues. Bone marrow transplantation has been successful, for example, in treating thalassemia, sickle cell disease, and immune deficiencies; liver transplants have been performed for Wilson disease (Desnick, 1981; Friedrich, 1984). Transplantation is a serious prospect for only a small minority of potential patients, however. This is because current methods require tissue compatibility between the donor and the recipient, a rare event, and because the methods require highly risky treatments to prepare the patient to receive the transplanted cells or organs. A final disadvantage of transplantation is its extraordinary cost.

There are thus several existing and prospective treatment for genetic diseases that do not require direct gene replacement or supplementation, but all have limitations and many genetic diseases have ***no*** treatment. As one physician summarizes the status quo, "therapy of most genetic disorders is still ineffective and inadequate" (Friedmann, 1983).

Gene therapy of somatic cells will therefore probably prove technically superior to alternative treatments for selected patients with some disorders.

SEVERITY OF SYMPTOMS **AND PROGNOSIS**

The patient expected quality and length of life directly affect the potential benefit and acceptable level of risk of any medical or experimental intervention. Extremely serious disorders, such as Lesch-Nyhan disease and ADA and PNP deficiencies, have such poor prognoses that even small potential benefits are welcome and large risks may be acceptable to the patient and his or her family because they pale in comparison to continued life with the disease.

Some examples of diseases likely to be targets for gene therapy are noted by category in table 1. The number of patients likely to be treated are noted in table z.

DATA MONITORING

For clinical trials to be optimally productive of new knowledge, investigators must have mechanisms for following patients, and have a protocol for obtaining whatever tissues may be needed and for analyzing them. Advance thought about how data monitoring will be done and disclosure of what it will involve to the human research Subjects should be an important aspect of any human gene therapy experiments. Attention to data monitoring will thus be one requirement for approval to begin clinical trials.

INFORMED CONSENT

Assurance that informed consent will be freely and appropriately obtained is required for all ex-

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Table 1.—Examples of Diseases for Which Gene Therapy Might Be Considered

1. **Protocols** for human gene therapy in somatic cells expected in next several years:
   - immunodeficiency caused by adenosine deaminase or purine nucleoside phosphorylase deficiencies (ADA or PNP deficiencies)
   - Lesch-Nyhan syndrome (complete hypoxanthine-guanine phosphoribosyl transferase deficiency)
   - urea cycle defects caused by deficiencies of arginosuccinate synthetase (citrullinemia) or ornithine carbamoyl transferase (OCT, also known as ornithine transcarbamylase)
2. Might be attempted in foreseeable future:
   - phenylketonuria (as improvement on current dietary treatment)
   - familial hypercholesterolemia
   - defects of the urea cycle other than citrullinemia and OCT deficiency:
     - arginemia (arginase deficiency)
   - mucopolysaccharidoses and other defined metabolic defects:
     - Gaucher disease (some forms)
     - metachromatic leukodystrophy (arylsulfatase B deficiency type with little brain involvement)
     - Hunter syndrome (enzyme detectable in normal blood)
     - branched chain ketoaciduria (severe grades)
3. Farther off because protein expression may require regulation:
   - hemoglobinopathies: (see *Technical Note 5)*
     - sickle cell disease, hemoglobin SC disease
     - alpha and beta thalassemia
   - hormone production defects
4. Farther off because gene product may be easily available for administration (diminishing the need for gene therapy):
   - growth hormone deficiency; some other hormone production defects
   - hemophilias
5. Unlikely unless new discoveries provide clues on how to approach gene therapy:
   (Some may require germ line therapy because of access to tissue sites or immunologic problems with gene product.):
   - Tay-Sachs disease and other metabolic defects that primarily affect brain
   - cystic fibrosis
   - type 1A growth hormone deficiency
   - most diseases inherited in dominant pattern (e. g., Huntington disease, Marfan syndrome, achondroplasia, etc.)
6. May not be applicable:
   - chromosomal disorders:
     - Down syndrome
   - environmental and multigenic disorders:
     - hypertension
     - diabetes

"Cloned human gene available

SOURCE: Wissow, 1984.

Table 2.—Numbers of Patients Who Might Be Treated by Somatic Cell Gene Therapy in the Near Future

Disorder	Number of patients with the disorder
Adenosine deaminase deficiency	40 to 50 reported worldwide
Purine nucleoside phosphorylase deficiency	9 patients in 6 families reported worldwide
Lesch-Nyan syndrome	1:10,000 males, estimated 200 new cases in the United States per year
Arginosuccinate synthetase deficiency	53 cases reported
Ornithine carbamoyl transferase deficiency	110 cases reported

SOURCE: Stanbury, et al., 1983, as modified by OTA.

periments involving humans (Code of Federal Regulations, 1983). In the case of human gene therapy experiments, this will include disclosure of what can reasonably be expected about:

- risks of new mutations,
- possible effects on the germ line,
- reversibility of side effects in the patient, and treatment for them,
- relative costs of alternative therapies,
- relative risks and benefits of alternative therapies,

procedures that will be done to obtain clinical data on the gene therapy experiments, procedures for dropping out of the study, and assurance that it is the patient's right to do so.

All human experimental protocols should be reviewed by local Institutional Review Boards (IRBs), as is the case with all experiments involving humans. In the case of human gene therapy, however, the NIH recently revised the Guidelines for use of recombinant DNA to state that research proposals involving human gene therapy (proposed by institutions that receive Federal funds for recombinant DNA research) must be submitted to NIH for approval, in addition to local IRB review. These protocols will be reviewed first by a Working Group on Human Gene Therapy, then by the Recombinant DNA Advisory Committee, and finally by the NIH Director before ap-

proval to proceed is granted. One purpose of this special care is to further insure proper informed consent of patients electing to participate in gene therapy experiments. FDA also has the authority to oversee the adequacy of informed consent in clinical experimentation involving new therapeutic products, and this might include gene insertion technologies (Esber, 1984).

One special aspect of human gene therapy, the potential for wide publicity, may merit attention in the process of securing informed consent. Widespread interest in human gene therapy among scientific, religious, and government leaders in advance of its successful application suggests that the early clinical trials will be subject to potentially intrusive publicity. It is unlikely that government oversight bodies can assure the privacy of subjects who agree to participate in gene therapy experiments, and so acknowledgement of this risk may be necessary by investigators before commencing. Investigators may also need to anticipate responding to the demand for media information by developing mechanisms for channeling interest through hospital spokesmen, preparing families to deal with the press, and careful observation of privacy safeguards. The risk of media exposure is part of the process of informed consent, because this may prove to be the salient difference between gene therapy and other experimental medical techniques.

Issues that may arise from clinical application

If gene therapy moves through the early stages of development and reaches the stage of standard medical practice, several medical issues may emerge. None of these is different in kind from issues arising in connection with other medical technologies, but the context of the new problems would be different.

Medical malpractice

Issues related to malpractice may be raised by gene therapy if it develops into a routine medical technology. Physicians could be sued, for example, for failing to treat a genetic disorder. A patient who suffered an untoward side effect because of genetic changes induced by gene therapy might also bring suit. What would the standards of care for this technique be?

Several medicolegal issues might enter into assessments of liability and responsibility, It is not clear, for example, who would be qualified to employ the sophisticated techniques of gene therapy if it were to become standard medical practice. Should all physicians do it? Only those certified by the American Board of Medical Genetics, the National Board of Pediatrics, the Hematology and Oncology subspecialty board in internal medicine, or the American Board of Obstetrics and Gynecology? Should gene therapy take place at all hospitals, or only in certain ones? Who would practice gene therapy, and where, may well be determined by decisions made by the court system, State and local Governments, national medical specialty boards, and other medical and legal organizations.

Parental responsibilities

Parental views on religion and medical practice, including those that might preclude even somatic cell gene therapy, might pit the beliefs of parents against standard medical practices. Many court decisions about whether to allow blood transfusions to children of parents who reject such treatments on religious grounds exemplify this kind of conflict. Some legal scholars have even contended that parents who fail to intervene on behalf of the health of their children might be forced to do so. In one recent case, a woman who objected to cesarian section on religious grounds was compelled to undergo the operation to preserve the life of the fetus (Lenon, 1983; Finamore, 1983). If gene therapy were widely available and standard medical practice, analogous conflicts might arise.

Whether medical practitioners, courts, institutional committees, or parents decide on who is treated will depend on how gene therapy and

other medical technologies are handled by the courts or in new legislation at the State or Federal level.

patents and trade secrets

The techniques involved in gene therapy involve the use of recombinant DNA to clone and insert human genes. The early applications, if they involve the diseases listed in table Z, are unlikely to involve patentable agents or processes, because the methods under development have been openly published and developed at several centers, and the recombinant DNA involved is available in several laboratories. Eventually, however, the complexity and variety of approaches to gene therapy might result in products or processes that could be patented. Patents might be sought, for example, for genetically altered viruses designed to deliver the human gene to the target tissue or that permit controlled expression. The criteria for granting such patents will be patentable subject matter, novelty, utility, and nonobviousness, the same ones used for other recombinant DNA products (OTA, 1984, ch. 16). The public policy issues of fair access to the technology and encouragement of innovation would also be analogous to those for other medical technologies.

A few distinctive aspects of patents and trade secrets are especially relevant to gene therapy. The review process by the National Institutes of Health (NIH) for approving experiments involving proprietary information might require closed sessions, so that trade secrets were not disclosed publicly. The guidelines for human gene therapy formulated by the NIH (described below) are not binding on private firms that do not receive Federal research funds, although companies would be likely to seek NIH approval in any event to avoid adverse publicity and to assure due process for questions that arise about liability and insurance. Finally, the flow of scientific and clinical information to other investigators might be inhibited if trade secrets related to gene therapy must be protected.

Insurance

Gene therapy might eventually be covered by standard medical insurance, or it might require special provisions. Gene therapy, if it follows the model of other medical treatments, will not be covered by insurance companies until its efficacy has been established for its intended application. Coverage by insurance will likely depend on the particular disorder, the relative cost (for gene therapy and the alternatives), and the safety and efficacy of the techniques involved.

Social implications of gene therapy

Gene therapy, should it prove useful, would be like other technologies in changing the character and kinds of decisions that individuals make. It would provide new options for medical therapy and imply new responsibilities for making such decisions fairly and for the benefit of both individuals and society. In the view of many religious and ethical thinkers, gene therapy restricted to somatic cell corrections of single gene traits differs little from other medical therapies (Neale, 1983; World Council of Churches, 1983; Siegel, 1982; Fletcher, 1982, 1983a, and 1983b).

There are risks and benefits associated with beginning gene therapy, as with any new technology. Public policy, public education, scientific and technical advance, and other factors can all influence which applications are pursued and which eschewed. In an open and democratic society, new technologies are greeted by different social groups in different ways. Some may believe that beginning gene therapy too closely resembles “playing God” or is too dangerous, while others impatiently await its application to the disease affecting a loved one.

Background

The application of gene therapy to humans is likely to be regarded throughout society as a significant step, whether done in somatic or germ

line cells. It will be a focus of attention because it is unprecedented and technologically sophisticated, and because it permits alteration of something considered fundamental to each individual—his or her genetic constitution. While genetic changes have been technologically induced for years—for example, in the use of some vaccines--the changes have never been so premeditated nor so direct as deliberately inserting new human genes to cure a specific disease. As noted above, however, the main difference between gene therapy and other medical technologies may be perceptual more than actual. The risks and benefits of gene therapy are analogous to those for other therapies, and many believe that it presents no fundamentally new ethical problems, yet there remains a gnawing discomfiture with the prospect.

In the absence of gene therapy after birth, an individual has no role in the choice about which genes he or she carries, and so bears no responsibility for carrying them. Once gene therapy is available, this may not be the case, and individuals ma-y play some role in selecting their genes. This prospect is frightening to many because new choices bring new responsibilities; new technologies can be misapplied. The magnitude of the responsibility is, to a large extent, determined by the power of the new technology. If, as suggested above, gene therapy is not widely applied in the near future because of limitations on the range of diseases to which it is applied, then the social impact of gene therapy is likely to be less than that associated with many other accepted medical practices.

Most of the major social impacts of genetic knowledge will almost certainly derive less from gene therapy than from genetic screening or other genetic testing. Some fundamental choices about privacy of data on patients' genetic constitution must be made as the new technologies provide greater amounts of such information (see app. B). The new information will, however, not be directly related to developments in gene ***therapy***, but rather to diagnostic evaluations of patients' predispositions to genetic diseases or special health risks.

Some fear that increased knowledge about how genes work may further promote a cold, abstract, and mechanistic view of human life. To the extent that this is true, however, it does not relate directly to gene therapy but rather to genetics in general, and even more broadly to all of science.

Social aspects of gene therapy that are mentioned below fall into several general categories:

- What process will determine when to begin gene therapy?
- How important are evolutionary considerations? and
- What might be the impacts on social institutions?

Major social issues

WHAT PROCESS WILL DETERMINE WHEN TO BEGIN GENE THERAPY?

The process of deciding when to begin experimental human gene therapy includes several components. Some judgments are technical, involving assessment of the expression of the gene of interest, for example, and such decisions are left to scientific peers to examine experimental design or determine which studies are relevant to a proposed project. Other judgments involve assessment of quality of life for a particular patient; such decisions can only be made by the patient, his or her family, the physician, or others who are familiar with the details of a particular case. Other judgments may involve determination of acceptable risk to society, and these invite wider public participation.

Many of the questions raised will be answered only in the context of a particular patient in a particular family seen by an individual physician, and the judgments of the parties most directly affected will decide the case within the constraints set by laws, regulations, and local ethics and human research committees. The context for making individual decisions will thus depend on peer review and compliance with human subjects guidelines. The criteria for peer review and setting of guidelines involve, in turn, government agencies that must ensure fairness, completeness, and representation of diverse and often conflicting viewpoints.

Judgments about whether a given experiment conforms to the criteria will differ among individuals. Some of the differences will reflect life experiences. A physician accustomed to treating cancer patients will have different views from a scientist whose primary interest is developmental biology. A hospital attorney may hesitate to endorse an experiment that the parent of an affected child would eagerly embrace. One suspicious of technology in general might reject experiments involving any level of risk.

Some urge caution in approaching uses of gene therapy.

> Once we decide to begin the process of human genetic engineering, there is really no logical place to stop. If diabetes, sickle cell anemia, and cancer are to be cured by altering the genetic makeup of an individual, why not proceed to other 'disorders': myopia, color blindness, left-handedness? Indeed, what is to preclude a society from deciding that a certain skin color is a disorder? . . .
>
> With human genetic engineering, we get something and we give up something. In return for securing our own physical well-being, we are forced to accept the idea of reducing the human species to a technologically designed product. Genetic engineering poses the most fundamental of questions. Is guaranteeing our health worth trading away our humanity? (Rifkin, 1983, pp. 232-233).

In contrast, an urgent request for support of gene therapy research is found in the words of Ola Huntley, three of whose children suffer from sickle cell disease:

> I resent the fact that a few well-meaning individuals have presented arguments strong enough to curtail the scientific technology which promises to give some hope to those suffering from a genetic disease. I have faith to believe that genetic therapy research, if allowed to continue, will be used to give life to those who are just existing . . . .I, too, would like to ask the question, who do we designate to play God? Aren't those theologians and politician; playing God? Aren't they deciding what's best for me without any knowledge of my suffering? I am very angry that anyone would presume to deny my children and my family the essential genetic treatment of a genetic disease . . ..1 see such persons as simplistic moralists who probably have seen too many mad scientist horror films. It's like saying that someone can deny others the right to drive or ride in an automobile because there is an ever-present danger of an accident (Huntley, 1983, pp. 166-169).

Such conflicting views cannot be assuaged by empty assurances, and public policy decision will typically be made without consensus. There are dangers in premature application balanced against undue delays of useful medical benefits. Public policy will be decided amidst great uncertainty. As one doctor noted, "the ethical principle that physicians have to be concerned about is that we know what we're doing before we promise that we're going to try and treat someone" (Ryan, **1983, p.** 172). In deciding when to begin experiments on human gene therapy, the need for further knowledge must be weighed against the benefits that might accrue to patients with severe and fatal diseases.

Most of the social and ethical questions raised about gene therapy could also be raised in the context of other medical interventions, such as use of antibiotics or acceptance of surgery. It is not the questions that are new, but rather a new technology that forces their reconsideration. Disagreement about the seriousness of the new social and ethical consequences of using gene therapy in humans hinges on incompatible judgments of how widely it will be used and how revolutionary will be its perceived impact on how humans view their own sanctity. Most scientists and clinicians believe that gene therapy will be only a small incremental medical advance applicable to a few patients, while religious and social commentators may reflect on its cumulative effects over generations. The general interest in human gene therapy has led some scientists and medical providers to urge caution so as to avoid political reaction against gene therapy among the general population (Rosenberg, 1983; Grobstein, 1984).

Public policy will have to be based on consideration of patient welfare, social impacts, religious precepts, and political realities. There is little reason to believe that differences in opinion about the appropriateness of human gene therapy will resolve spontaneously, or even after extensive public discussion. Where there is no agreement

on what decision to make, the only alternative is a process for making the decision, and government agencies must demonstrate that the process is rational and fair (Bazelon, 1983).

Wide public discussion and agreement on a process do not guarantee fair decisions or correct assessments of risk and benefit. Errors of judgment may occur even with unassailable expertise and completely democratic participation. Resort to fair and open process is not, therefore, perfect, but merely the best practical solution **to** assure fairness.

Given the anticipated public interest in and controversy about human gene therapy, any successful mechanism for permitting its commencement will involve a public process including discussion among individuals with different informed perspectives. Such discussion may arrive at consensus, but if it does not, documentation of the fairness and rationality of the decisionmaking process will be the only practical course. The Federal Government will be involved in decisions about human gene therapy because of its involvement in medical research, health care, and issues that attract wide public interest.

There are several Federal agencies already in place that can educate the public and make decisions about when to begin human gene therapy. These include the Recombinant DNA Advisory Committee of the NIH, the Food and Drug Administration, and several other bodies within the department of Health and Human Services. These will be described below in the section on the Federal Role in Gene Therapy.

HOW IMPORTANT ARE EVOLUTIONARY CONSIDERATIONS?

Direct manipulation of the genome inspires visions of mankind controlling its own evolution, depleting the diversity of genes in the human population, and crossing species barriers to create new life forms. The magnitude and rapidity of change caused by direct genetic intervention, however, are likely to be far smaller than the large effects caused by relaxing historic selection pressures on the human population through changes in the environment, sanitation, and health care.

Discussion of ***germ line*** gene therapy is most relevant to permanently changing the human gene pool because it would lead to inherited changes. At present, however, such discussion is necessarily vague and speculative because the technology does not exist and may never be used. There will doubtless be continued public interest in ensuring fair and open debate on whether human germ line gene therapy would be appropriate. It is impossible, however, to make estimates of the potential magnitude of its impact on human populations now.

The effects of somatic cell gene therapy will depend on how many patients receive such therapy, and to which conditions it is applied. It is not possible to make firm predictions about how many patients might eventually be treated by gene therapy, because it is not now certain that even somatic cell gene therapy will prove medically useful. The effect that somatic cell therapy would have on human population genetics would be no different in kind than that from other technologies that affect the patient and do not lead to inherited changes, Most of the changes would be due to preservation of the lives of those who would otherwise die before reproducing, the same effect that results from diet therapy in PKU, or clotting factor replacement in hemophilia.

While it is not possible to estimate the number of patients that might eventually be aided by somatic cell gene therapy, it is possible to estimate the impact of correcting those genetic defects that are currently targeted. These will be the potential genetic impacts that must be assessed by those approving the early experiments in gene therapy. As can be seen in table 2, the diseases for which gene therapy is now contemplated are quite rare. The total number of patients with these conditions that might be treated using somatic cell gene therapy would likely be less than 300 per year in the United States, and would probably be far fewer until the technology were accepted. This figure compares to the approximately 4 million births in the United States each year.

Changing the Gene Mix in Human Populations.—Somatic cell gene therapy would have no direct effect on the mix of genes in human popula-

tions, and would have only the indirect effects noted above. Germ line therapy, in contrast, would alter the prevalence of some genes, although the magnitude of such effects is impossible to predict because so many factors are involved.

Direct germ line gene therapy of recessive disorders would, for most diseases, have a noticeable effect on human evolution only if widely practiced for hundreds of generations. The number of generations needed to have a significant effect would depend on the type of gene being corrected, its prevalence in the population, when the disease were expressed (in adulthood or childhood), the severity of the disease caused by it, and many other factors. If gene therapy were used only to treat single gene recessive traits, then it would take several hundred generations measurably to alter the prevalence of the gene in the population. For defects that are present on one percent of chromosomes in the human population, for example--corresponding to a genetic disease much more common than any under consideration for gene therapy now—it would take 1,500 years to increase the frequency to 2 percent.[14] If germ line gene therapy were widely practiced for a large number of diseases, including common dominant traits, then alterations might be noticed much more quickly, but such applications are not now envisioned.

Depletion of Diversity in the Gene Pool.-– There is excellent evidence that some genetic diseases are common because of an advantage conferred to those individuals who carry ***one*** copy of the aberrant gene. Those who carry one copy of the sickle cell anemia gene, for example, are better able to combat malarial infections. The genetic disease is the price paid to preserve this advantage for the population on average, mitigated only by the statistical rarity of having two abnormal genes (and thus the disease) (Vogel, 1979).

[14]This example is based on discussion of eliminating rare genes for recessive disorders in several references (Li, 1961; Vogel, 1979). These assume that those who carry two copies of a defective gene would not reproduce. In considering the impact of human gene therapy for those who would otherwise die before reproducing, the situation is reversed but the time scales would be comparable,

Genes causing other genetic diseases may also serve a purpose that has not been discovered, and so elimination of such genes might prove deleterious to the human population in the long run. In somatic cell gene therapy, the patient own genes would not be deleted, but new information would be added in such a way that it would not be inherited. This would have no impact whatever on the population's reproductive gene pool. If gene therapy permitted the survival of patients who would otherwise die, however, then genes causing diseases might slowly become more widespread because they would not be eliminated.

Even if gene therapy did have an effect on genetic diversity, this might not prevent its use, The risk of slightly reducing diversity in the entire human population would likely seem insignificant to those patients for whom the potential benefits loom large and immediate. Perpetuation of genetic disease, particularly of the severe childhood diseases that are now the targets for gene therapy, would seem a cruel means to an end of uncertain import.

The sickle cell example is instructive in this sense, as well. While it is widely accepted that the sickle cell gene conferred certain advantages in combatting malaria among Mediterranean populations, it is also true that current antibiotics and sanitation technologies have been much ***more*** effective in protecting the same populations. In the era of modern medicine, sickle cell disease is no longer a necessary price to pay for genetic protection from the ravages of malaria.

The arguments for refraining from gene therapy in order to maintain genetic diversity are also weakened when raised in a population whose main long term problem may be the very rapidity of its growth. When a population is rapidly expanding, the diversity of genes generally increases because there are more individuals who can carry new genes.

Crossing Species Barriers. -Recombinant genetic technologies permit genes from one species to be inserted into another. In the animal experiments cited, for example, rat growth hormone genes were put into mice and rabbit globin genes

into rats. It is unlikely that an animal gene would be used for human gene therapy, because if an animal gene is available, then isolation and cloning of its human counterpart would be routine. Human genes will be used in animals, however, to test the safety and efficacy of gene therapy before it is tried in humans. What would be the significance of using human genes in animals?

Mythology and literature contain numerous examples of hybrid creatures that combine the characteristics of man and beast or involve engineering completely new organisms (Capron, 1984c; Siegel, 1982). One need only think of the minotaur (the apocryphal man-bull hybrid of Crete who devoured fair youths from ancient Greece), the golem (a creature of Jewish lore created to protect the residents of Prague; the golem eventually turned against them and had to be destroyed), or Frankenstein's monster to note the horror associated with semi-human creatures, It is widely accepted in the religious and professional ethics communities that attempting to create such creatures would be immoral (World Council of Churches, 1982; National Council of Churches, 1984; Siegel, 1982,1983); it is also impossible to create such creatures by attempting to alter single gene defects. Some of the issues raised by interspecies transfer of genes are further discussed in Technical Note 3,

FETAL RESEARCH

Research involving human fetuses is a topic of controversy in the United States, and 25 States have statutes that limit or prohibit it (Andrews, 1984b; Quigley, 1984). Fetal research bears on gene therapy primarily if germ line gene therapy is considered. If germ line gene therapy on human embryos is to be undertaken, it must rest on a foundation of knowledge about development and genetic expression in very early human embryos. Such knowledge can only be obtained using such embryos.

Even if germ line therapy is not considered, there may be instances in which fetal research would be useful in establishing safety or efficacy of somatic cell gene therapy. The history of research on Rubella during the 1960s may illuminate the utility of fetal investigation in several respects.

Concern about Rubella infection, particularly its proclivity for causing congenital malformations, intensified following the epidemic of 1964, It was well known that Rubella infection during pregnancy could cause malformations, but the mechanisms were not clear. Investigation of the epidemic was advanced by research on fetuses that either spontaneously aborted or were aborted because an infected woman chose to avoid the risk of bearing a deformed child. Fetal research showed that a majority of fetuses in women known to be affected had been directly infected by the Rubella virus, that the deformations were likely due to direct fetal infection, and that fetal infection often persisted long after the woman was no longer symptomatic (Horstmann, 1965).

Fetal investigation also led to the development of Rubella vaccines. Many vaccines were developed during the mid-1960s, including the RA 27/3 vaccine derived from an infected human fetus and propagated in tissue culture of human cells (Plotkin, 1965; Plotkin, 1969). This strain is now the only Rubella vaccine licensed for use in the United States (Plotkin, 1981).

Finally, the guidelines for use of Rubella vaccines were influenced by human fetal research. Animal experiments showed that Rubella could infect fetuses of pregnant females (Parkman, 1965), as was expected from human studies. Preliminary experiments in monkeys, however, did not show fetal infection by the weakened Rubella used in vaccination (Parkman, 1966). The number of monkeys tested was necessarily small because of the expense and difficulty of animal experiments, and investigation of humans proved necessary. Scandinavian workers showed that in contrast to the monkey experiments, vaccine strains might infect the human fetus (Vaheri, 1969). These experiments could only be done on aborted fetuses. The findings were considered in drafting the recommendations for use of vaccines in pregnant women (Recommendations, 1969).

The strains of vaccine now in use are different from those used in the Scandinavian experiments, and further research on current strains (involving women who have inadvertently been vaccinated during pregnancy) has demonstrated that

the risk of fetal infection from Rubella vaccination during pregnancy is quite low (Plotkin, 1981).

Fetal research thus played a role in better understanding the congenital Rubella syndrome, in development of vaccines, and in establishing safe practices for human vaccination. An analogous role in establishing scientific background and testing safety and efficacy of gene therapy might also require fetal research for some future applications.

There is no reason to test human gene therapy protocols in human fetuses now because neither fetuses nor pregnant women are contemplated for treatment. Should this change, then tests involving fetuses would be desirable. If a need for application to fetuses or pregnant female patients emerges, then it may depend on study abroad (where fetal research is practiced), relaxation of fetal research guidelines in the United States, or repeal of statutes in those States that prohibit such research (if the research is to be conducted in such States). This issue will be especially difficult to resolve if gene therapy is shown useful for severe diseases of early childhood. This is because gene therapy that is useful in infants is likely, in some cases, to be potentially even more beneficial during fetal development–before the metabolic abnormalities caused by the genetic disease have caused any deformities or irreversible effects on the nervous system.

WHAT MIGHT BE THE EFFECTS ON SOCIAL INSTITUTIONS?

Several religious leaders have noted that gene therapy may be one more factor tending to reduce perceptions of humanity to mechanistic interpretations (Zaner, 1982; Siegel, 1982, 1983; World Council of Churches, 1982; National Council of Churches, 1984). Focus on mechanism may lead to diminished attention to social and moral values, and may threaten attitudes about the sanctity of human life. The effects of the new technology on attitudes are not certain, however, and the same commentators note that appreciation of the complexity of life may increase our regard for life more than it attenuates it. The attempt to save lives by gene therapy is itself an attempt to preserve or improve particular lives. The specific effect of gene therapy in changing perceptions is, in any case, likely to be one small part in the general growth of science, complementing other fields that also alter our self-perceptions such as neuroscience, computer science, psychology, evolutionary biology, ecology, and other parts of biology and medicine. If gene therapy is found medically useful, it may prove difficult to deny benefits to needy patients on the basis of long-term shifts of human self-perceptions.

Gene therapy may play a larger role in indirectly altering parental expectations. If genetic therapy is successful for extremely serious diseases, then it might be applied over time to progressively milder medical problems. This prospect raises the possibility that parents may more and more expect “perfect” children. So long as gene therapy is confined to disorders that are recognized as significant burdens, then it will merely bean addition to the medical armamentarium. If it becomes possible to treat more and more disorders, especially if attempts are made to affect intelligence or physical traits, then gene therapy might indeed raise concern about parental expectations of their children. Again, however, the definition of appropriate application is one that must be widely discussed because it is more a social than a medical issue (although medical factors are highly relevant). Discussion of such potential dangers is, given present technology, mere speculation for now; as the technology develops, public discussion may need to be encouraged if it appears that gene therapy is becoming widely applicable.

The Federal role in gene therapy

The Federal Government performs several functions that may affect the development and application of human gene therapy. Most biomedical research is supported by the Federal Government through the National Institutes of Health (NIH) and other Executive agencies. Regulation of

pharmaceutical products is the responsibility of the Food and Drug Administration (FDA). Genetic services including manpower training, basic and applied research, genetic screening, and counseling, are partially supported through block grants given to individual states under authority of the National Sickle Cell Anemia, Cooley's Anemia, Tay-Sachs and Genetic Diseases Act (Reilly, 1977), and administered under the Omnibus Budget and Reconciliation Act. Finally, the Federal Government, through its legislative, judicial, and Executive branches, is often an effective instrument for public discussion and education, through the Department of Health and Human Services, congressional hearings and activities, and such agencies as the President's Commission.

International interests in human gene therapy

Human gene therapy is widely regarded to be closer to clinical testing in the United States than any other country. Other developed nations will soon follow, however, and international interest in its development has been noted, primarily in Canada and Europe. Canadian research groups have been involved in the design of viruses that might be used in gene transfer (Merz, 1984), and several European government groups have made statements related to gene therapy. The Parliamentary Assembly of the Council of Europe, for example, made a recommendation that '(the rights to life and human dignity . . . imply the right to inherit a genetic pattern which has not been artificially changed," although this right was explicitly qualified so as to "not impede development of the therapeutic applications of genetic engineering (gene therapy), which holds great promise . . . " (Parliamentary Assembly, 1983). The Parliamentary Assembly also called for the development of a list of diseases that could be treated using gene therapy, based on several criteria:

- seriousness of the disease,
- simplicity of the technique and applicability to only single gene disorders,
- application to a well characterized disease,
- supervision by scientific and ethical review commit tees,
- restriction to centers of demonstrated expertise, and, interestingly,
- exclusion of genes that are "the object of commerce ."

A recent report on reproductive technologies was submitted to the Parliament of the United Kingdom by a committee headed by Dame Mary Warnock. The report recommended that a new governmental licensing agency be created to oversee embryonic and fetal research and its applications. The committee also briefly commented on potential germ line gene therapy, and recommended that the licensing authority give "guidance on what types of research, apart from those precluded by law, would be unlikely to be considered ethically acceptable in any circumstances" (Committee of Inquiry, 1984). The licensing authority would thus monitor gene therapy research and consider whether germ line therapy should be permitted.

European political history in dealing with genetic technologies differs from that in the United States. The United Kingdom, for example, has approached the regulation of novel biological technologies from a different perspective (Wolstenholme, 1984). Fetal research is now performed in the United Kingdom and Australia, and so questions regarding its regulation are more prominent there than specific applications to gene therapy. In the United States, fetal and embryonic research has not been federally funded for almost a decade (see below), and the scientific and medical focus of gene therapy has been on ***somatic*** cell therapies whose development does not entail the use of fetuses or embryos.

Federal agencies potentially involved in gene therapy

Several Federal agencies potentially have purview over some aspect of human gene therapy. The National Institutes of Health, as the primary sponsor of relevant research and the location of the Recombinant DNA Advisory Commission, is involved in approving both research grants to do gene therapy research and in overseeing compliance with Federal research guidelines.

The National Institutes of Health (NIH), through its Recombinant DNA Advisory Committee (RAC), is currently the most active Federal body involved in monitoring human gene therapy. It was established in 1974 and is charged with recommending guidelines for safe conduct of research involving recombinant DNA (or, by extension, RNA) (Milewski, 1984). The RAC has established a Working Group on Human Gene Therapy, whose members are listed in appendix C, to develop guidelines for research on human applications of gene therapy. The Working Group plans to have guidelines published in 1985, in anticipation of proposals for human gene therapy, The Working Group shall evaluate research proposals received by NIH, and shall report to RAC. RAC shall, in turn, report the the Director of the NIH, who will then approve the proposal or suggest needed alterations. Another function of the Working Group will be to educate the public and to review some broader social implications of human gene therapy that are not included in review by local Institutional Review Boards (Working Group on Human Gene Therapy, 1984).

The Food and Drug Administration (FDA) will also play a role in regulating some aspects of human gene therapy. The FDA has the authority to regulate drugs, including biological products intended for use in the diagnosis, treatment, or prevention of diseases or injuries in humans. The FDA will become involved in human gene therapy if it involves products such as nucleic acids or genetically modified viruses that are subject to agency regulations (under authority of the Food Drug and Cosmetic Act and the Public Health Service Act) (Miller, 1983a). The role of the FDA generally includes review of applications submitted for products used in investigational studies and encompasses the manufacture and quality control procedures applied to such products. The FDA review includes evaluation of the design of clinical and preclinical studies, adequacy of procedures for assessing safety and efficacy, and methods for obtaining informed consent from patient participants (Miller, 1983b).

The FDA authorizes (by approval of a New Drug Application or granting of a license) the marketing of products when a review process has concluded that the data obtained during investigational trials support the safety and efficacy of the product for its intended labeled claims (Miller, 1983b).

In addition to the NIH and FDA, which are already monitoring human gene therapy, there are several other Federal agencies or bodies that might become involved in the future.

An Ethics Advisory Board (EAB) is an entity composed of non- government experts in ethics, law, medicine, and others with expertise related to a particular topic under consideration. One such board was formed in 1979 to advise the Secretary of Health and Human Services on several topics, most notably fetal research. Federal regulations state that “One or more Ethical Advisory Boards shall be established by the Secretary” (Code of Federal Regulations, 1983) yet no such board exists at present. An EAB was intended to “render advice consistent with the policies and requirements . . . as to ethical issues)” (Code of Federal Regulations, 1983). Such a board, if it were now reconstituted, might play a role in coordinating and overseeing the Federal Government’s activities regarding human gene therapy, including public education, supervision of NIH, FDA, and other agencies in the Department, and advising the Secretary on other actions. Consideration of the broader questions related to progress in human gene therapy would fall within the mandate established for EABs.

The Federal Interagency Advisory Committee on Recombinant DNA Research, established in 1976, is another group that has not played a direct role in human gene therapy, but could theoretically do so. The Committee is composed of members from several Federal agencies involved in activities related to recombinant DNA research. Members of the Committee agreed to comply with the NIH Guidelines in 1976, thus in effect transferring authority to NIH for biomedical research and clinical investigations. Recently, other agencies, including the Department of Agriculture and the Environmental Protection Agency (both of which have members on the Interagency Committee), have become involved in regulating agricultural and environmental applications of recombinant DNA research. The Committee may thus play a more active role in agricultural, environ-

mental, and other new areas of research, but it is likely that most authority to monitor and regulate human gene therapy will remain at NIH and FDA because these agencies have the most extensive experience with biomedical and clinical applications.

The Office of Science and Technology Policy (OSTP) is an Executive agency, headed by the President Science Advisor, that reports directly to the President. The OSTP has taken a lead in Federal oversight of some areas of science and technology, and has recently coordinated a group of government officials in dealing with the questions surrounding deliberate release of genetically altered organisms into the environment, and other novel applications of biotechnology. The OSTP could conceivably also serve a similar function for gene therapy, although the extensive experience of FDA and NIH in questions relating to health and medical technologies makes OSTP less likely to be involved in human gene therapy than in more general questions such as environmental release or new agricultural applications.

Determination of the Federal role in monitoring and public debate about questions relating to bioethics, including human uses of recombinant DNA technology, was a focus of considerable legislative activity in the 98th Congress. Bills to reauthorize the lapsed President's Commission were introduced in both houses, but no further action on those bills was taken. Representative Gore proposed a new President's Commission on Human Applications of Genetic Engineering that eventually became part of the House version of the NIH authorization bill. Senators Hatch and Kennedy proposed creation of a bioethics commission at OTA as part of legislation creating a new National Institute of Arthritis and Musculoskeletal and Skin Diseases at NIH. The Senate and House bills were referred to conference. The conference report authorized a new Biomedical Ethics Board, composed of 6 Senators and 6 Representatives, and a Biomedical Ethics Advisory Committee, composed of 14 appointed individuals and experts in relevant disciplines. The Committee would have performed studies related to bioethics, including two mandated studies: one on fetal research and another on human applications of genetic engineering (including human gene therapy) (Conference Report, 1984). The legislation reported from conference was passed by both houses, but vetoed by president Reagan on October 30, 1984. The future of a Federal body for investigation of bioethical questions is thus uncertain.

Functions of the Federal Government

SUPPORT OF RESEARCH

The Federal Government, through the National Institutes of Health (NIH), is the primary sponsor of biomedical research in the United States. The NIH budget for 1983 was $3.8 billion, accounting for 36 percent of all funds spent in the United States on health-related research (NIH, 1984). In those areas of biological science related to human gene therapy, the NIH funds the bulk of research, although a few companies with expertise in biotechnology are known to be sponsoring some research relevant to gene therapy.

The relative rarity, scientific difficulty, and long term investment necessary to develop gene therapy for any one genetic disease suggest that research may not occur unless there is public funding. Individual genetic diseases are thus "orphan" disorders when taken singly, yet relatively common as a group. The technology to identify or treat one genetic disease often suggests means for approaching biochemically similar disorders, and many aspects of research on on one disorder may be directly applied to others. A recent example of this phenomenon is the discovery that the gene for Huntington disease is located on human chromosome 4. This discovery was made by applying a technique developed for general mapping of the human chromosomes to large families in the United States and Venezuela[15] (Gusella, 1983; Wexler, 1984; Rosenfeld, 1984; Kolata, 1984a). The same technique, which may permit earlier diagnosis and eventual identification of the specific gene responsible for the disease, promises to apply to many other genetic diseases. The financial and scientific investments in discovering

[15] The technique called restriction fragment length **polymorphism linkage** analysis, **was** developed to locate genes even when **the** gene had not been cloned or even identified (Botstein, 1980, 1984). This method for identifying the chromosomal location of genes is described in app. A.

and developing a technique used in locating the Huntington disease gene may thus also pay off for other disorders.

Research on genetic diseases is likely to continue to depend heavily on Federal funding, and so long as gene therapy remains experimental, Federal research policy will be influential in its development. Seminal discoveries related to human gene therapy will likely derive from both clinical research and basic research on molecular genetics and biochemistry. The technologies of recombinant DNA and gene transfer now contemplated for use in human gene therapy are themselves results of basic genetic inquiry, and further practical applications of basic research are likely to emerge. This has been the pattern of development of molecular biology and other biomedical sciences-research in one area leading to breakthroughs in an unexpected and seemingly unrelated discipline. The discovery of DNA's relationship to inheritance was itself such a serendipitous discovery, resulting from Avery's work attempting to identify why certain bacteria caused pneumonia (Thomas, 1984; Judson, 1980).

Research on developing animal models of human genetic diseases may be important in facilitating human gene therapy applications. Such models provide methods for testing the efficacy and safety of treatment methods.

In addition to basic research, some early experimental trials in humans will likely be supported by Federal funds. Decisions about how Federal research funds are expended for research on basic molecular genetics, animal models of genetic diseases, and preliminary human applications will thus directly affect how rapidly gene therapy develops and which diseases will be addressed.

REGULATION OF MEDICAL APPLICATIONS

A research proposal involving recombinant DNA is generally originated by a scientist working at a university, industrial laboratory, or other research center, A research proposal includes general background, goals of the experiment, methods to be used, evidence for efficacy, provisions for assuring safety and informed consent of patient participants (and may also include information on compliance with standards for animal care). The proposal is sent to local review committees that assess its compliance with safety and human subjects protection guidelines. Certain classes of experiments are automatically referred to NIH for approval, and cases that cannot be decided locally are also referred to NIH.

These procedures are the ones followed by scientists and clinicians using Federal funds who act in good faith. Human investigations supported by private firms must also meet human subjects protections guidelines, usually to avoid problems of liability and insurability. Clinical investigations of pharmaceutical products, including genes or modified viruses used in treatment, must also be submitted for FDA review.

Ensuring Compliance with Human Subjects Protections.—A process for protecting human subjects in research already exists. In the context of experiments involving human gene therapy, a proposal for an experiment involving human subjects should be sent to an institutional review board (IRB), a local committee that would then review the proposal for compliance with human subjects protection standards, according to the following criteria:

> minimization of risk to the subjects,
> reasonable risks in relation to anticipated benefits,
> equitable selection of subjects,
> assurance of informed consent,
> adequate provisions for monitoring data,
> provisions for protecting patient privacy, and
> assurance that decisions to participate in research will not be coerced (Code of Federal Regulations, 1983).

Approval by a local IRB will be required before proposals are forwarded to NIH for approval. IRB approval may be contingent on approval by the NIH. When received at NIH, the proposal will be published in the Federal Register for public comment and will also be referred to the Working Group on Human Gene Therapy, which will then report to the RAC for review. If the proposed experiments meet the standards of the RAC, then they are referred to the NIH director for approval (Working Group on Human Gene Therapy, 1984).

Ensuring Safety and Efficacy.—Mechanisms for reviewing research proposals to ensure safety potentially fall under the authority of several groups. Assurance of safety is analogous to human subjects protection, including review by NIH and FDA after approval by local safety and human subject committees. Each investigator must submit his research proposal to his or her local Institutional Biosafety Committee (IBC), which assesses compliance of the proposed experiments with NIH safety guidelines for recombinant DNA research. In the case of human gene therapy, the risks and benefits of proposed experiments will also be reviewed, followed by approval by the NIH and FDA before commencement (Krause, 1984, p. 17847).

There are several weaknesses in this regulatory schema. Only research conducted at institutions accepting federal funds for recombinant DNA experiments are obligated to conform to the NIH Guidelines by law, although to date private research groups have voluntarily submitted to RAC Guidelines. (Private corporations have complied at least in part because of the risk of public censure, potential loss of insurance coverage, and possible added legal liability in civil suits if they do not.) The formal penalty for not conforming to NIH guidelines is denial of Federal research funds to the institution submitting the proposal. This is quite powerful for universities and most research centers, but is not a direct economic incentive for compliance in some privately sponsored research.

Another feature of the current review process is the lack of evaluation of research goals. IRBs are specifically precluded from assessing the '(long-range effects of applying knowledge gained in the research" (Code of Federal Regulations, 1983). This is quite appropriate in the context of a particular experiment involving patients with specific defects, and IRBs cannot be expected to do more than investigate specific protocols. The lack of purview over goals, however, leaves a vacuum for determining which experiments are contrary to public policy. The NIH has formed the Human Gene Therapy Working Group in part to fill this vacuum, but there are potential questions of conflict of interest because **NIH** is also the primary sponsor of biomedical research. Assessment of public policy on goals for research, including human gene therapy, could be performed by an EAB, congressional commission or other Federal body.

In addition to review of research proposals on human gene therapy by the NIH, the Food and Drug Administration (FDA) also has authority over human experiments involving therapeutic products. Genes introduced for gene therapy could constitute such a biological product under FDA jurisdiction and would thus involve FDA approval before commencing (Miller, 1983b). FDA oversight would follow regulator procedures used for other products: submission of evidence for safety and a rational basis for introduction of the product into humans (stemming from animal experiments, in vitro studies, and relevant previous clinical trials), Investigator submissions must include data showing that the product is adequately pure and sufficiently potent to justify clinical trials (Office of Biologics Research and Review, 1983). The FDA then evaluates the evidence and determines whether risk and benefit considerations support clinical trials.

FDA authority may, in some circumstances, overlap that of the NIH, whose Guidelines explicitly provide for oversight of human gene therapy and experiments that involve recombinant DNA (or molecules derived from rDNA).

Whatever the mechanism or agency involved, protocols and products will be evaluated case by case. This will certainly involve local IRBs, NIH, and FDA, and may eventually include other Federal agencies as well. If individual applications of human gene therapy becomes standard medical practice, or even widely available, they will then be governed primarily by professional standards, civil suits, or local authorities, like other medical technologies.

For early experiments on human somatic cell gene therapy, present oversight methods that involve local IRBs, RAC, NIH, and FDA appear adequate. For more controversial applications of gene therapy involving germ line alterations, wider public discussion, open goal setting, and greater government oversight may prove necessary to avoid undue controversy and assure prudent public policy.

PAYMENT

If gene therapy were to become incorporated into routine medical practice, the Federal government might become involved in paying for its use. As long as gene therapy is experimental, most costs will be borne by research funds. Typically, as a therapy is used more widely, funding becomes much more complex. Many regulatory decisions are made about reimbursement at the Federal and State levels, and individual insurers make reimbursement decisions that are subject to State and Federal regulations.

Medicare reimbursement of gene therapy might apply, for example, to those instances (probably quite rare) involving people over age 65 or who suffer from chronic kidney disease that could be treated by gene therapy (polycystic kidney disease is a dominant trait that leads to kidney failure, but is not now a candidate for somatic cell gene therapy because the gene has not been identified and its mechanism of causing disease is not understood).

Medicaid is joint State and Federal health program that pays for medical services provided to indigent individuals. Medicaid reimbursement would involve both State and Federal policy, and might be used to pay for gene therapy of pediatric patients in indigent families.

Little has been written about how to pay for gene therapy. If other medical technologies are taken as examples, early costs are likely to be relatively high, and drop as clinical experience and technical innovations accumulate. Decisions will be made about applications to specific disease entities rather than for gene therapy in general, and there will likely be regional and institutional variation among payers as to which applications are reimbursable. Mechanisms of payment could range from complete public subsidy to total payment from personal income at each stage of development. If gene therapy proves successful in its early applications, more attention will need to be devoted to sources of payment.

PUBLIC EDUCATION AND DISCUSSION

The high level of interest in topics relating to genetics suggests that mechanisms need to be developed that permit discussion at all levels of society. Several issues relating to genetics, such as practices in a particular laboratory or individual patient-physician decisions, must be made locally. Other issues of national importance, such as research policy, health policy, and civil rights, may require attention by the Government and international agencies.

Careful public policy decisions about novel technologies require an educated public. Federal agencies have been directly involved in educating the public about gene therapy, through congressional hearings such as Human Genetic ***Engineering*** held by the Subcommittee on Investigations and Oversight of the House Committee on Science and Technology in November 1982, symposia such as the Public Forum on Gene Therapy sponsored by NIH in October 1983, and publications such as Splicing Life issued by the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research.

Several scientists have expressed concern that the nuances of genetic technologies, such as the distinctions between somatic and germ cell manipulations, may not be completely understood by the public (Baltimore, D., in Friedmann, 1983, p. 59). One basis for such concern is the experience with the early debates about the safety of recombinant DNA, when laboratory research involved precautions and preservation of detailed records on laboratory safety of recombinant DNA work that were considered onerous by some scientists (Weissmann, 1981). Rancorous public debates occurred before the City Council of Cambridge, Massachusetts, and other places about whether certain recombinant DNA research should be permitted (Wade, 1984). While recognizing the need for caution in research on recombinant DNA, some believe that public concern led to overly stringent regulation triggered by baseless fears. One scientist noted, "It seemed that we had lost track of the serious scientific and health considerations and were operating in a climate of hysteria–some of which passed for responsibility" (Leder, 1984).

Public education is, many believe, the best solution to misapprehensions about genetic technologies (Beckwith, 1984; Capron, 1984a, b). Increased public education was designated a high priority by President's Commission, and was in-

eluded as the first of the major functions of any Federal agency overseeing the development of genetic technologies (President's Commission, 1982, pp. 82-84). The consensus on a need for public education does not, however, necessarily imply agreement that public education is primarily a function of a Federal oversight body (H. I. Miller, 1984).

Equitable social policy is another reason to foster public discussion. The governmental role in developing, regulating, and applying gene therapy is crucial, as noted in other sections of this Background Paper. Informed public decisions presuppose not only adequate knowledge, but also a process for ensuring that all views are fairly represented.

The need for wide public discussion of human uses of gene therapy and other genetic technologies has been noted by religious groups (World Council of Churches, 1982; National Council of Churches, 1984), by the President's Commission (President's Commission, 1982), by ethicists and scientists (Grobstein, 1984), and in congressional hearings (Gore, 1982). Opinion on this issue appears to have converged from many quarters, involving scientists, ethicists, politicians, and religious leaders, and resulting in what one observer has called an "amazing consensus" about the need for continued oversight and discussion at the Federal level (Nightingale, 1984). The functions of such discussion include definition of goals, identification of public policy issues, inclusion of conflicting views held by different constituencies, and consideration of short- and long-term consequences of genetic interventions of concern to various scientific, medical, religious, and consumer groups.

There are some potential problems that even an effective Federal forum for discussion may not accomplish, however. It is doubtful that any commission can resolve the differences that emerge from moral and social plurality in the United States. For example, what conditions should be treated by gene therapy? Disorders such as baldness or short stature that are considered minor annoyances by one person might merit somatic gene therapy as judged by another.[16] No regulatory apparatus is suited to resolving such dilemmas. Public debate can air differences, but should not be expected to eliminate them.

In addition, there is a danger of gratuitous additional regulation that would impede the development of legitimate human applications of genetics if new agencies are created, or overly stringent regulations imposed 0-I. I. Miller, 1984).

Finally, public debate cannot and should not intervene in the decisions best made by individual patients and the health professionals who provide care. Personal choice is a value that should be constrained as little as possible in establishing public policy.

FAIR DISTRIBUTION OF BENEFITS AND COSTS

The costs and benefits of gene therapy are uncertain because the technology is in its infancy. If gene therapy becomes a part of routine medical practice, however, then many issues relating to distribution of costs and benefits may arise. In general, these would be similar to those raised by other medical technologies: payment, informed consent, and fair access. Fair distribution of costs and benefits would be one of the considerations in reimbursement decisions mentioned above. It may fall to government to rectify reimbursement decisions that do not provide equal access to all social sectors and ethnic groups. Access to gene therapy by the indigent or by minorities especially prone to certain genetic diseases, for example, might prove of special concern.

Decisions made now about research funding will also influence the future distribution of benefits from gene therapy. Because different genetic diseases are more common in some racial groups, decisions about which diseases to investigate can be expected to influence the later availability of gene therapy or other treatments among such groups. Neglect of hemoglobin disorders, for example, would be of more concern to Blacks and those of Southern Mediterranean extraction than to other Caucasians. Federal decisions about which diseases are addressed in genetic research thus have potential distributional consequences, and the large share of genetic research supported by the Federal Government makes such decisions important in determining which populations may eventually benefit from available technologies.

[16] Neither of these conditions is sufficiently understood to be a candidate for somatic cell gene therapy. They are mentioned only to illustrate a point, not to indicate technical feasibility.

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PROTECTION OF INDIVIDUAL RIGHTS

Some individual rights protected by the Federal Government may be influenced by gene therapy, as by any new medical technology. Any threat to such rights is, however, more likely to derive from new diagnostic techniques of genetic testing than from gene therapy. Maintaining the privacy of genetic diagnostic information about disease risks is likely to be a much larger problem for individuals' rights than performing gene therapy because: 1) more people will be affected, 2) more information will be generated by diagnostic techniques than therapeutic interventions, 3) the diseases for which genetic risk factors might be assessed are common and have large economic consequences for employers and insurers, and 4) problems in protecting individual rights for gene therapy are quite similar to the problems arising from other therapies, while genetic diagnostic technologies may make much more information available of a new type. These issues are briefly addressed in appendix B.

Knowledge that a person has undergone gene therapy should be accorded the same privacy safeguards applied to other medical information. In addition to ensuring the privacy of genetic information, including medical information about gene therapy, the Federal Government has accepted a role in protecting the interests of research subjects. Such protections include IRB's and, in the case of gene therapy, the RAC at NIH. FDA oversight also includes attention to informed consent of participating patients.

A few weaknesses persist in the present methods of research subject protection. Children and mentally incompetent patients cannot consent to treatment because they cannot understand the consequences of such consent. The process of informed consent requires different standards in different court jurisdictions (see app. B and Andrews, 1984a), but all standards involve a competent patient or surrogate decisionmaker who can rationally balance risks and potential benefits. In cases of disagreement with physicians or other health professionals, families often are involved in making decisions in the best interests of the patient. In some instances, especially when there is disagreement between medical professionals and families, it is not clear who can and who cannot give consent for treatment or participation in experiments. The problem of surrogate informed consent is especially likely for gene therapy, because many genetic diseases primarily affeet children or cause mental incapacity in adults. There are special guidelines for IRB's to consider in approving research protocols that involve children (Code of Federal Regulations, 1983). Uncertainty about informed consent can act as an impediment to research on the one hand, and may leave some patients insufficiently protected on the other. Some states are drafting legislation to deal with the problem (Andrews, 1984a). State and local initiatives may eventually clarify the legal status of surrogate informed consent, but, in the interim, responsibility for monitoring the informed consent process for research participation will fall to IRB's and the courts.

Case histories

IN VITRO FERTILIZATION

Some lessons from Federal policy relating to research and clinical applications of in vitro fertilization (IVF) may be applicable to the development of gene therapy technologies. In vitro fertilization is the process of obtaining sperm and eggs from donors, uniting the gametes in the laboratory, and implanting the products of fertilization in a woman's womb. This technology was developed in the 1950s, and first successfully applied to humans in 1969. Improvements in fertilizing eggs in the laboratory led to the first human applications of in vitro fertilization a decade later: Louise Brown, a normal infant conceived using in vitro fertilization, was born on July 25, 1978. She has been followed by more than 700 pregnancies resulting from in vitro fertilization and embryo transfer (Hodgen, 1984).

The primary intent of those using in vitro fertilization in humans is to permit infertile couples to have children (Hodgen, 1984) although other applications are technically possible.

In vitro fertilization is related to gene therapy because, for technical reasons, attempts at germ line genetic alterations are most likely to be attempted on early embryos. Germ line gene therapy would involve either extraction of a fertilized embryo from a woman (before the embryo had

implanted in the uterine wall) or, more likely, in vitro fertilization either immediately preceded or followed by addition of genetic material. Availability of in vitro fertilization is thus a precondition for successful germ line gene therapy (Ryan, 1983) and so policy affecting in vitro fertilization practices will also affect germ line gene therapy.

Even if in vitro fertilization were not directly related to gene therapy, the history of Federal policy on it would still be of interest because it is a controversial biological technology analogous to gene therapy in some respects. A brief review of decisions made about in vitro fertilization may highlight potential pitfalls that could also occur in connection with gene therapy.

There has been a de facto moratorium on Federally sponsored research on human in vitro fertilization in the United States since 1975. There are nonetheless at least 60 centers and 200 programs offering it in the United States (Abramowitz, 1984; Hodgen, 1984). The research leading to these early efforts was performed primarily in the United Kingdom and Australia. American centers have adopted the technology developed in other nations, or have treated patients using private moneys paid by patient fees.

Congress imposed a temporary moratorium on Federally sponsored human in vitro fertilization research in 1973, after NIH received its first request for a grant for fetal research. The 13 month moratorium was technically lifted in 1975, when guidelines proposed by the Ethics Advisory Board (EAB) of the Department of Health and Human Services (then the Department of Health, Education, and Welfare) were published. The guidelines sanctioned carefully constrained research, provided that strict procedures were observed, including:

> the intent of the research was to improve understanding of fertilization and assess risks, the information could not be obtained by other means,
> informed consent, including disclosure of risks, was obtained, and other regulations on human subjects research were observed,
> embryos beyond the fourteen-day stage of development were excluded if embryos were not to be implanted back into prospective mothers,
> measures were taken to ensure that possible risks to the public were disclosed,
> only gametes from married couples were employed if embryos were implanted in prospective mothers, and, most importantly,
> approval was obtained from the EAB, in addition to IRB review, before commencing.

The findings of the EAB have never been accepted by a Secretary of HHS (or HEW), the EAB has been disbanded, and no Federal grants have been approved for research on in vitro fertilization. The NIH authorization bill from the 98th Congress, as passed by both houses and vetoed by the President, would have mandated a further 3 year moratorium on human fetal research, and the new congressional bioethics board would have undertaken a study of it (Conference Report, 1984). The moratorium on human fetal research will continue, however, until an EAB that could approve it is reconstituted by the Secretary of Health and Human Services.

The Federal moratorium on research in the United States did not prevent the development of in vitro fertilization technology or its clinical application, although its development has probably been somewhat slowed (Abramowitz, 1983). There is some concern that the technology has developed with less than usual Federal oversight, and that some desirable steps, such as testing in non-human primates, have been skipped in the transition from experiments in lower mammals to human clinical applications (Ryan, 1983). Experiments have not been subject to the NIH peer review process, and may have "circumvented systematic accumulation of knowledge" (Ibid., p. 152). The Federal Government may have lost some ability to monitor and control the technology by failing to sponsor research (Ibid., pp. 151-153) or at least to provide a mechanism for Federal oversight. Furthermore, the technology developed in spite of the lack of a consensus about its moral acceptability (Ibid., p. 153).

The unusual development of in vitro fertilization research is exemplified by one technique of in vivo fertilization of an egg in one woman fol-

lowed by transfer of the embryo to another. The technique permits obtaining the fertilized egg without subjecting the donating woman to a major surgical procedure. This technique has been developed with corporate funds in the United States, and those who sponsored the research have applied to patent some of the instruments involved, as well as the process itself (Annas, 1984; Chapman, 1984). A patent for a medical procedure is unusual, although not unprecedented (Brotman, 1983); if granted, it would give the sponsoring corporation the ability to limit the application of surrogate embryo transfer to those who obtained a license. Such limitation might increase costs and diminish access to the technology, but might also permit enhanced quality and controlled diffusion of the procedure. One of the arguments used in favor of patenting the process is that the research was privately sponsored, and so the investors merit a return on their investment (Chapman, 1984; Annas, 1984).

The example of in vitro fertilization technology shows that techniques developed in other countries can be imported, and such applications made available in the United States, even in the absence of Federal research support. Widespread clinical use of in vitro fertilization also shows that technologies whose appropriateness is seriously questioned may nevertheless enter clinical practice without extensive Federal oversight or regulation, and in the absence of pervasive public discussion.

Gene therapy is different from in vitro fertilization because there is no moratorium on gene therapy research, and so the bulk of research is funded, like other biomedical research, through the Federal government. Such research necessarily falls under the oversight purview of NIH, and consequently the RAC and its Human Gene Therapy Working Group. There are many agencies with jurisdiction over gene therapy, including local IRBs, NIH, and the FDA (for specific products). These bodies are now preparing to deal with the incremental medical advance embodied in ***somatic*** gene therapy. Review by these bodies may not be adequate for extension of gene therapy to reproductive cells. Several authors refer to the need for national public discussion of the greater ethical and social implications raised by ***germ line*** alterations before commencing such research (although the authors do not uniformly suggest that such discussion necessarily take place through the Federal Government) (Fletcher, 1983b; Grobstein, 1984; Nightingale, 1984). The lack of a forum for conducting public debate holds also for fetal research and in vitro fertilization.

Human gene therapy may be less attractive to corporate investors than in vitro fertilization research. The investment incentives for gene therapy are diminished by the relatively small number of individuals with any given genetic disease. This restriction does not hold, however, for all diseases and does not necessarily preclude the development of profitable products. Gene therapy applicable to certain diseases such as sickle cell anemia or cystic fibrosis might have a market large enough to justify corporate interest. In addition, a general approach to gene therapy that could apply to many genetic disorders might be patented, analogous to the Cohen-Boyer patent for recombinant DNA, or kept as a trade secret. The incentives for private investment may thus be weaker than for in vitro fertilization, but may nonetheless be sufficient to induce corporate research and development.

There is a prominent regulatory difference between in vitro fertilization and human gene therapy: in vitro fertilization is not clearly under the jurisdiction of FDA or NIH, but human gene therapy is subject to both. Gene therapy is likely to involve new pharmaceutical products, and hence be regulated by FDA, because experiments will involve introduction of new genes or modified viruses into human cells or into patients. In contrast, in vitro fertilization is more a process than a product. Further, in vitro fertilization is applied to correct infertility, a problem that is not necessarily considered a disease or injury, and thus may not fall under FDA purview. In vitro fertilization has passed through the early phases of technological development to clinical application with little regulation or Federal oversight, but human gene therapy is receiving extensive public scrutiny and Federal oversight despite its technological infancy.

EARLY ATTEMPTS AT HUMAN **GENE THERAPY**

The Rogers Cases.—Between 1970 and 1973, Dr. Stanfield Rogers, an American, assisted a German physician in treating three sisters with the

genetic disease arginemia. The sisters were infected with the Shope papilloma virus, which had activities that physicians believed might supplement an enzyme activity missing in the three girls. The treatment was unsuccessful.

The Shope virus experiments were performed before ethics review boards, IRB's, or IBC's existed. The experiments were discussed openly, although much of the debate about their propriety did not take place until after the clinical trial. The debate centered on whether there was sufficient evidence to anticipate patient benefit, and whether the intervention had been undertaken at a time when it could best benefit the sisters (Fletcher, 1983). The ethical debate about the Shope virus experiments is thus unresolved, although it is clear that no institutional or legal precepts were violated.

The Cline Cases.—Martin Cline, an American scientist and physician primarily working at the University of California at Los Angeles (UCLA), became the first investigator to attempt gene therapy using recombinant DNA in 1980, when he attempted to treat two patients who had thalassemia. One patient was treated in Italy, and the other in Israel. Dr. Cline withdrew samples of bone marrow from each of the patients, treated them with DNA containing a normal hemoglobin protein gene, and restored the treated bone marrow cells to the patients. The process for returning the bone marrow involved killing a portion of the native cells by radiation, so that the treated cells would have a location in which to grow. The experiment was the first attempt at somatic gene therapy using recombinant DNA techniques.

At the time the experiments were performed, approval by the local review committees was pending. The gene therapy experiments were attempted on July 10 and July 15, 1980, and Dr. Cline's proposal to the UCLA Human Subject Protection Committee was disapproved on July 16 (Talbot, 1982). Dr. Cline had prior approval for a gene therapy experiment by the local board in Israel, but not for the one, involving recombinant DNA, that he actually performed.[17]

In contrast to the Shope virus experiments, there was a consensus that Dr. Cline's experiments were premature and unethical. Dr. Cline resigned his division chairmanship, and the NIH terminated two grants, To prevent future abuses, NIH also added several requirements, including the need to submit an assurance of compliance with human subjects safeguards, prior review by the local IBC and NIH of all recombinant DNA experiments, and inclusion of the NIH report of the events to the review groups for his subsequent new applications for NIH grants (Talbot, 1982). The special sanctions were in effect until May 1984.

The issues raised by the Cline experiments are likely to recur in any debate about the propriety of human gene therapy, and so a summary of the justifications and objections is instructive, followed by a review of Federal policy in the Cline clinical trials.

There were several justifications for undertaking clinical trials of human gene therapy, as noted in previous sections. Those used to justify the experiments involving the patients with thalassemia included:

- The condition was irreversible.
- Alternative therapies were unpleasant, expensive, led to deleterious side effects, and did not cure the cause of the disease, but merely diminished its effects (Wade, 1980; Cline, 1982).
- The Human Subjects Protection and Institutional Biosafety Committees had been considering the proposals in the period between May 1979 and July 1980 without approving or disapproving them. There was also an apparent logjam, with the Human Subjects Committee requiring that the IBC approve the protocol before it would assess it, and the IBC awaiting the review of the Human Subjects committee. Attempts to refer the matter to the RAC were thwarted because NIH refused to consider the proposals, reasoning that the human subjects aspects were much more im-

[17]The Israeli board had approved insertion of genetic material that included the normal hemoglobin protein genes. Dr. Cline contends that the use of recombinant form was a technical detail that did not add to the danger of the experiments, because the genes tend to combine in the cell even if they are not in recombinant form when first inserted (Cline, 1982),

portant than the recombinant DNA technology itself (wade, 1981a).

The Israeli experiments were approved by three committees in Israel, although not for the protocol involving ***recombinant*** DNA (Wade, 1981b).

Those who criticize the Cline experiments do not disagree with these facts, but interpret them differently, and add the following considerations:

- The patients selected had an irreversible disease, but were not in a terminal state (as called for in the protocol). They were alive more than two years after the experiments were undertaken, despite lack of any benefit deriving from the experiments (Cline, 1982).
- The human experiments were never published and were based on other animal experiments that had not been peer-reviewed at the time (and about which there are disagreements regarding interpretation) (Cline, 1982).
- There were no data on the safety of the procedure, because directly analogous experiments had not been attempted in animals (Williamson, 1982).
- Dr. Cline personally decided to deviate from his protocol, using a recombinant molecule rather than separated genes. While this decision may have been scientifically valid, Dr. Cline failed to notify the Israeli committees, committees in the United States, and even the patients and his collaborators, of his decision to use ***recombinant*** DNA (Wade, 1981a; Cline, 1982).
- The ambiguities about which committee should first approve the protocol had been resolved by the time the experiments took place. The decision to refrain from using recombinant DNA removed the need for IBC approval, leaving only the local Human Subjects Protection Committees to approve the protocol (Wade, 1980).
- The Human Subjects Protection Committee in the United States was not dallying, but awaiting expert comments from four consultants to assess the scientific basis of the experiments. The process took time, and the comments were passed on to Dr. Cline and his collaborators as they were received; the investigators knew that there were objections to starting the experiments (Wade, 1980).

The issues raised by the controversial Cline experiments point out the importance of Federal research policy decisions. The research in question was funded, in large part, through NIH, and the review procedures for application to humans were specified by the NIH. The sanctions rendered against Dr. Cline were imposed by the Department of Health and Human Services, based on NIH review; many believe that one reason that the sanctions were relatively stringent was because of congressional concern about previous laxity on the part of NIH in punishing those who violated research guidelines (Sun, 1981; Wade, 1981 b).

Some of the consequences of the Cline experiments are less tangible than receipt or denial of grant applications. Many believe that the Cline experiments are one reason for the current prominence of gene therapy in the debate about recombinant DNA. Critics of the technology may cite Dr. Cline's experiment in arguing for tighter restraint on scientists because they cannot be trusted to behave responsibly (Wade, 1980).

A de facto moratorium on somatic and germ line gene therapy has reigned since 1980. The Cline experiments may have catalyzed formation of a consensus that the time was not ripe for such experiments (Walters, 1982), and the opprobrium directed at Dr. Cline may have made scientists aware of the public sensitivity of the issue. The case, above all, highlighted the changing milieu for making decisions about human subjects in clinical research, and the growth of research oversight by the Federal Government. The results have been summarized by John Fletcher, a specialist in bioethics at NIH:

> Dr. Rogers treated the German sisters before prior group review became institutionalized. Dr. Cline, on the other hand, attempted to bypass that safeguard by withholding information from those who passed judgment on the wisdom of the experiment. The censure falling on Dr. Cline because of his deception indicates the strength of prior group review as a structure to guide somatic gene therapy when it becomes feasible (Fletcher, 1983b).

Conclusion

The first realistic applications of human gene therapy will be closely scrutinized by both the public and the Federal Government. Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies. Whether somatic cell gene therapy will become a practical medical technology will thus depend on its safety and efficacy, and the major question is when to begin clinical trials, not whether to begin them at all. The quality that distinguishes somatic cell gene therapy most strongly from other medical technologies is not technical, but rather the public attention that is likely to attend its commencement.

Federal oversight mechanisms for research and clinical application of *somatic* cell therapy are already in place, and enforcement of the mandated approval processes has already taken place in one instance, the breach of NIH guidelines perpetrated by Dr. Martin Cline. Committees exist at local institutions to monitor protocols for human subject protection, and all proposals for federally sponsored clinical trials should be referred to the RAC at NIH for approval, and may also be reviewed by FDA.

The consensus about the propriety of somatic cell therapy does not extend to treatment for traits that do not constitute severe genetic diseases, and does not encompass germ line gene therapy in humans. The question of whether germ line gene therapy should ever begin is now highly controversial. The risk to progeny, relative unreliability of the techniques for clinical use, and ethical questions about when to apply it remain unresolved. The question of whether and when to begin germ line gene therapy must therefore be decided in public debate informed by technological developments.

If gene therapy develops as a viable new medical technology, issues will emerge regarding who is to pay for it, how to assure equitable access to it, who is qualified to perform it, how to regulate its proper use, and which diseases merit its application. Many Federal agencies, including NIH, FDA, and health care payers, will be involved in such issues if the technology becomes part of standard medical practice.

Technical note 1

DNA function

Deoxyribonucleic acid (DNA) is a long, double stranded, helical molecule that contains building blocks (nucleotide bases) in a sequence which encodes instructions for all the metabolic processes in the human body, These range from growth and development through specific biochemical interactions involved in the digestion of food and synthesis of new molecules. DNA regulates its own expression and controls the production of proteins: structural proteins, **used to build the framework of cells, organs, and tissues; and enzymes, used to perform biochemical activity. There are two major processes involved in putting this information to use in the body-transcription and translation.**

Transcription is the simplest of these two processes. It consists of making an RNA (ribonucleic acid) copy of the DNA. This copy is then used to transport the instructions from the DNA to the protein building apparatus in the cell, outside the nucleus. This RNA copy of the DNA is called '(messenger RNA" (mRNA) because the message it carries from the gene allows the construction of the specified protein.

The process by which the mRNA is formed is very simple, taking advantage of the unique properties of nucleic acids (DNA and RNA). The double stranded DNA molecule separates, or unzips, at which point specific proteins present in the nucleus (enzymes) recognise precise signals present in the DNA (e.g., sequences of nucleotide **bases, such as TTAA) and attach to the DNA at those sites. One enzyme (called an RNA polymerase) then moves along the DNA molecule, constructing an mRNA molecule that has a complementary sequence of nucleotide bases (i.e., where there is an A in the DNA, the mRNA polymerase will add a T to the growing polymer). The end result is an RNA molecule that is a mirror image of the DNA region, or gene, which can then direct the assembly of a specific product.**

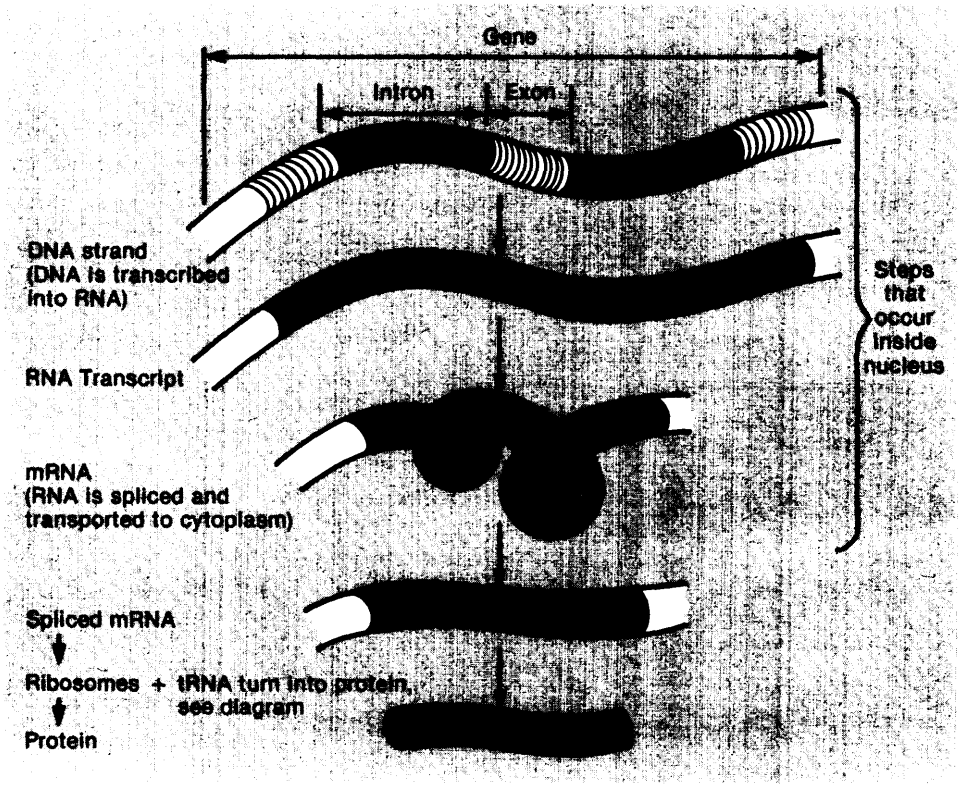
Before this mRNA molecule is used to assemble a protein in the process of translation, however, it must first be subtly modified; trimmed and tucked, as it were, so as to more precisely fit its function. There are several of these trimming processes and they are known altogether as "mRNA processing" or "post-transcriptional modification". Our understanding of these processes is incomplete, but the two best known are "excision/ligation" and "methylation. "

Excision/ligation is most similar to an editing process, and it is necessary because many genes contain more nucleotide bases than are necessary to code for the number of amino acids the finished protein will contain. Within a given gene there are two types of regions: "exons," **or expressed regions, and** '(in= trons, " or intervening regions. Exons contain the information that precisely directs the assembly of the protein product, that is, the sequence of amino acids added to the growing protein chain during translation. Introns, on the other hand, are the regions found between expressed regions. Their function is unclear; one hypothesis is that introns are involved in regulating gene expression (which includes turning genes on and off and controlling the number of mRNA molecules produced, and therefore the amount of gene product). The original mRNA transcript is thus trimmed and spliced by specific enzymes and transported from the nucleus to the cytoplasm, where it is decoded or translated into protein (see diagram).

Methylation refers to the process of attaching a small molecule (a methyl group, or a carbon with three attached hydrogens, CH3) to the backside of one of the nucleotide bases in the mRNA. The reason for this is not completely understood, but it is thought that methylation alters the mRNA in **such a way that some enzymes responsible for degrading it will not do so as quickly as they otherwise might. Methylation provides a method for controlling the longevity of mRNA molecules; it is desirable for some to be very short lived (where only a small amount of the encoded protein is required, as for an enzyme briefly needed) and for others to last longer (e.g., the mRNA coding for hemoglobin production in red blood cells, which live for about three months in the bloodstream).**

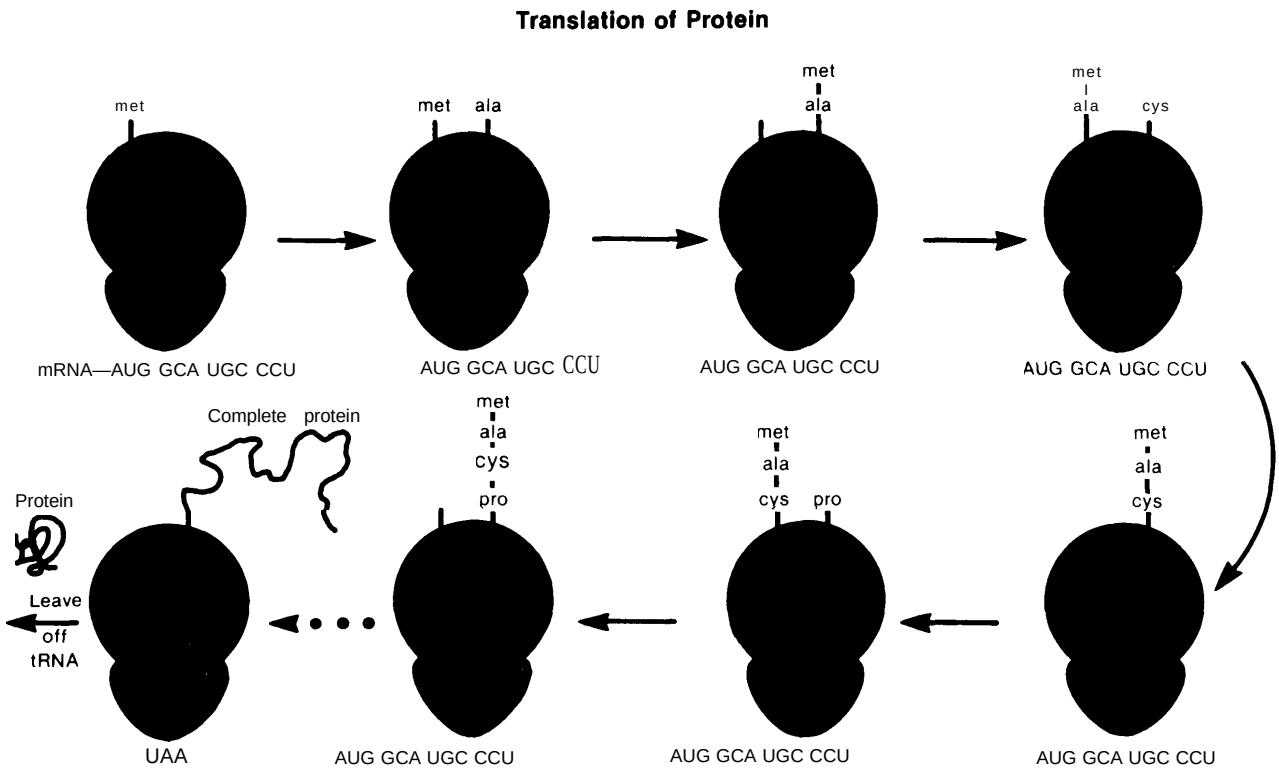
The second major process involved in making use of the information encoded in the DNA is translation, This takes place after mRNA is transcribed from DNA and then transported from the nucleus into the cytoplasm. In translation the information encoded in mRNA is decoded (translated) into protein by ribosomes. Ribosomes are complex structures within the cell that serve as the sites of protein synthesis, and they are composed of a number of different proteins combined with several different RNA molecules. On ribosomes, amino acids are joined one at a time to form a growing polypeptide chain. These individual amino acids are brought to the ribosome by transfer RNAs (tRNAs). Each different amino acid is brought

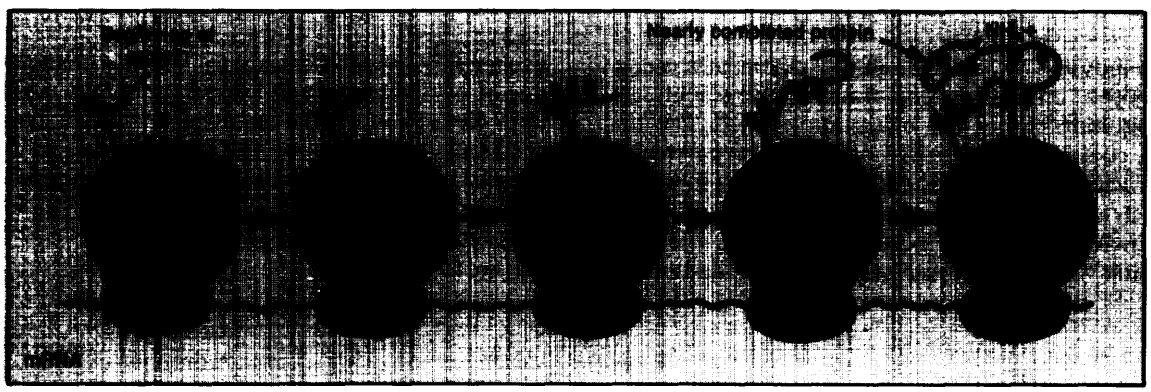
Gene Splicing



SOURCE: Office of Technology Assessment, adapted from Stanbury, et al., 1983,

to **the ribosome by a specific tRNA, which has a recognition site at one end specific for that amino acid, and a recognition site at the other end specific for the mRNA coding sequence that calls for the particular amino acid. These specific coding sequences in mRNA occur in a linear chain, and the amino acids added to the growing polypeptide as the mRNA moves along the ribosome reflect the linear sequence encoded in the master DNA region (gene) in the nucleus. Proteins produced by translation of messenger RNAs can begin their lives as enzymes involved in specific chemical reactions in the cell, or the proteins can be moved around or modified so that they become part of the surface of the cell, part of the cell's skeleton, or perform some other function.**





tRNA translates from mRNA into protein, with ribosomes acting as the conveyor belt to allow this process

SOURCE. Office of Technology Assessment.

Technical note 2

Genetic engineering techniques: cloning and vectors

There are several techniques of genetic engineering that are fundamental to efforts at human gene therapy. The most basic of these is cloning, or making multiple copies of a specific single gene. Once a gene has been cloned, it may be made in as many copies as desired (and thus easily studied), or moved from place to place through the use of specialized agents known as *vectors.* **There are several types of vectors; viruses** (bacteriophages, or phages), plasmids, or transposable elements.

Cloning involves several different steps. First the gene of interest must be identified; if it exists in only one copy per haploid genome (as with single gene, or Mendelian defects) then that one copy must be selected from perhaps as many as 100,000 other genes—a formidable task. As daunting as this problem is, however, there are some elegantly simple solutions.

The most favored of these is to identify the messenger RNA **used by ribosomes to assemble the protein of interest. Although mRNA is short-lived and notoriously delicate, this can often be done. From this mRNA a complementary DNA (cDNA) molecule can be synthesised, labeled with a tracer (radioactivity or a dye) and then used as a probe to identify the gene.**

If an mRNA cannot be identified, then it is possible to start with the protein product itself. This protein can be analysed and its amino-acid sequence determined. The amino-acid sequence can be used to deduce the nucleotide base sequence of the gene encoding the protein. A DNA molecule can then be synthesised and used as a probe to locate the relevant gene with its associated control sequences.

Once identified and located, special enzymes (restriction endonucleases or restriction enzymes) make it possible to isolate the entire intact gene and insert it into the appropriate vector. Plasmid (circular DNA molecules found in the cytoplasm of bacteria such as E. coli) or virus (phage) vectors make it possible to produce enormous numbers of copies of the gene of interest.

Plasmids.—In addition to the genetic information required for the existence of a simple bacterium, which is contained in its own genes, on its own chromosome, many bacteria also carry in their cytoplasm small circular molecules of DNA that replicate on their own. These are called plasmids and any number of them, from none to hundreds, can be found in individual bacteria. They are transmitted to progeny cells with the cytoplasm (hence the name) as the parent cell divides. The genetic information encoded in plasmid DNA often determines specialized characteristics of the bacteria, such as resistance to antibiotics. Their small size and simplicity have made them handy tools for the precise duplication and delivery of genetic information.

Some plasmids can be injected into the cells of higher animals where they replicate or integrate and pass from cell to cell as the cells divide. They are widely used in copying and multiplying genes because the special characteristics (e.g., antibiotic resistance) are easily engineered. These can he used to selectively promote the growth of cells that contain the plasmid, and thus also the desired genes.

Phage.—Phage (or bacteriophage) are viruses that infect bacteria, commandeer the bacterial machinery, and use it to translate the genetic information contained in the phage into phage products. Normally this leads to an infected bacterium producing phage offspring, but if the genes for building phage are replaced with a gene of interest to researchers, then the infected bacterium will produce copies of that gene instead. Phage can thus be used in much the same way that plasmids can, to make multiple copies of a given gene. The choice between using phage or plasmids as cloning vectors is based on the ease with which genes of different sizes or composition can be cloned with the different methods, and the advantages of different screening methods that can be used with the different vectors.

Transposable Elements.—Transposable elements (transposons) are relatively small molecules of DNA that can insert themselves into the genome of the host organism and move from site to site within it. Their origin is uncertain, but they seem closely related to some viruses. They have been called infectious or parasitic DNA and behave in some ways very much like infectious agents.

Genes of interest can be inserted into a transposable element, and thus be incorporated into the host genome along with the transposable element at specific sites. Although there are no transposable elements presently in use in human cells, they have been successfully used to "treat" genetic defects in fruit flies of the genus *Drosophila* (Rubin and Spradling, 1982; Spradling and Rubin, 1983). A mammalian equivalent to a transposable element would be a welcome discovery, as it could be used to control points of insertion into a human genome very precisely. Some viruses being considered as vectors for human gene therapy have similarities to transposable elements, including precise insertion sites.

Technical note 3

Violating species barriers

The majority of gene-therapy cases in humans would involve transplanting human DNA from one individual to another. In the forseeable future, research on and application of these techniques (or capabilities) is unlikely to use genes from an animal species to treat human genetic diseases. It is more likely that techniques involving the transplantation of genetic material from one animal species to another would be useful in agricultural or industrial applications; work of this kind has already been performed (involving human genes being moved into certain agricultural animals). The most far- reaching experiments of this sort are designed to increase understanding of mechanisms of genetic control and gene regulation. * This research will enhance scientists' ability to work with the genes of individuals within a species, and thus decrease the need to transfer genetic material between species in future therapeutic endeavors. The question has arisen, though, as to whether such work should be completely avoided or terminated because of an inherent danger or impropriety in "violating species boundaries." A look at nature offers a useful perspective.

A species is a community of organisms that is reproductively isolated from other such groups; that is, within a species there is interbreeding (exchange of genetic material) among individuals and their offspring, but none with individuals of other, different species. The problems with this widely used definition are several, and many of them are quite technical and esoteric. The most significant of these involve the existence and frequency of hybrids, or "crossbreeds" between species.

If species are to be defined on the basis of reproductive isolation, a sort of "genetic quarantine)" then violations of this quarantine, hybrids, should be rare and **unusual. This is emphatically not the case in nature. Hybrids are well known in higher organisms, where admittedly many are sterile (e.g., mules, resulting from a mating between a horse and a donkey). However in some groups hybrids between species are so common that distinct populations of these intermediate forms may exist along the distribution boundaries of neighboring species (Endler, 1977; Mayr, 1963, 1970). In these hybrid populations it is very difficult to assign an individual to either of the parent populations, which might themselves be quite easily distinguished from one another. Hybrids are more common in less advanced vertebrates, such as amphibians or fish, and there are even cases known where new species have been formed by hybridization between two previously existing species (White, 1978). In other situations species "boundaries" are so permeable that relatively widespread movement of genetic material from one species to another exists, a phenomenon called introgression. In plants, hybridization is so common that one leading expert (Raven, 1980) has concluded that it is almost useless to talk of "species" and that the most important reproductive group is the local population, or deme. To complicate matters further, some recent research offers tantalizing hints that horizontal transmission (between individuals of the same generation) very similar to the sort contemplated in some types of gene therapy have probably taken place between distantly related species (felids, or cat-like creatures, and primates) in the past (Benveniste and Todaro, 1982; Lewin, 1984). Specialists today are therefore becoming increasingly interested in factors that keep a species together rather than in mechanisms that may serve to keep them apart (Paterson, 1981, 1982).**

This is a useful approach in considering animal experiments relevant to human gene therapy. The question changes from "When can we justify violating species barriers?" to '(How much transmission of genetic material from one species to another can be tolerated before the integrity or separateness of the recipient species is threatened?" The answer is clear—an enormous amount; far more than would ever be involved in any case of gene therapy.

*The study of oncogenes (genes whose expression is linked to cancer) has involved hundreds of transfers of genes between species. From them we have learned enormous amounts about the mechanisms of carcinogenesis and gene regulation.

Technical note 4

Fertilization, implantation, and development

FERTILIZATION

When a sperm and egg (or ovum) meet, the sperm penetrates the wall of the egg. The genetic material from the sperm and egg unite, and the process of unifying the genetic contents of sperm and egg is called fertilization. The cell thus formed, containing DNA from both sperm and egg, is called a zygote. The mass of cells in the earliest stages after fertilization is also called a conceptus.

The release of an unfertilized egg from a woman's ovary is triggered by a burst of luteinizing hormone, or LH, from the pituitary gland (located near the base of the brain). The released egg migrates from the ovary a short distance through the abdominal cavity and into the oviducts, or Fallopian tubes. The Fallopian tubes lead into the uterus, and are the usual site of fertilization after the sperm have migrated into them from the vagina through the uterus to meet the descending egg. The developing conceptus then continues its descent through the Fallopian tubes into the body of the uterus.

CELL DIVISION

The zygote begins to divide, first into two cells, then into four, then eight, and so on. During the earliest stages of development all the cells are more or less equivalent. Once more than 16 cells are present, however, some distinctions between different types of cells begin to appear. Quite small and difficult to detect at first, these differences become more pronounced as cell division and growth continue, and form the foundation for the later differentiation of tissues and organs.

Different terms are applied to the developing organism as larger numbers of cells accumulate. The process of cell division is called cleavage. When enough cells have accumulated (between 32 and about a hundred), the term morula is used. The following stage, when the cells arrange themselves around a central cavity, is called the blastocyst. About 1 week after fertilization the blastocyst attaches to the uterine wall to continue further development.

IMPLANTATION

Implantation is the term applied to the process by which the conceptus attaches to the wall of the uterus and begins to send fingers of tissue (chorionic villi) into the wall of the uterus as anchors. These fingers are made up of embryonic cells that manufacture hormones to support pregnancy; they also form the network of supporting tissues that will eventually become the placenta, nourishing the developing embryo, and later fetus.

DEVELOPMENT

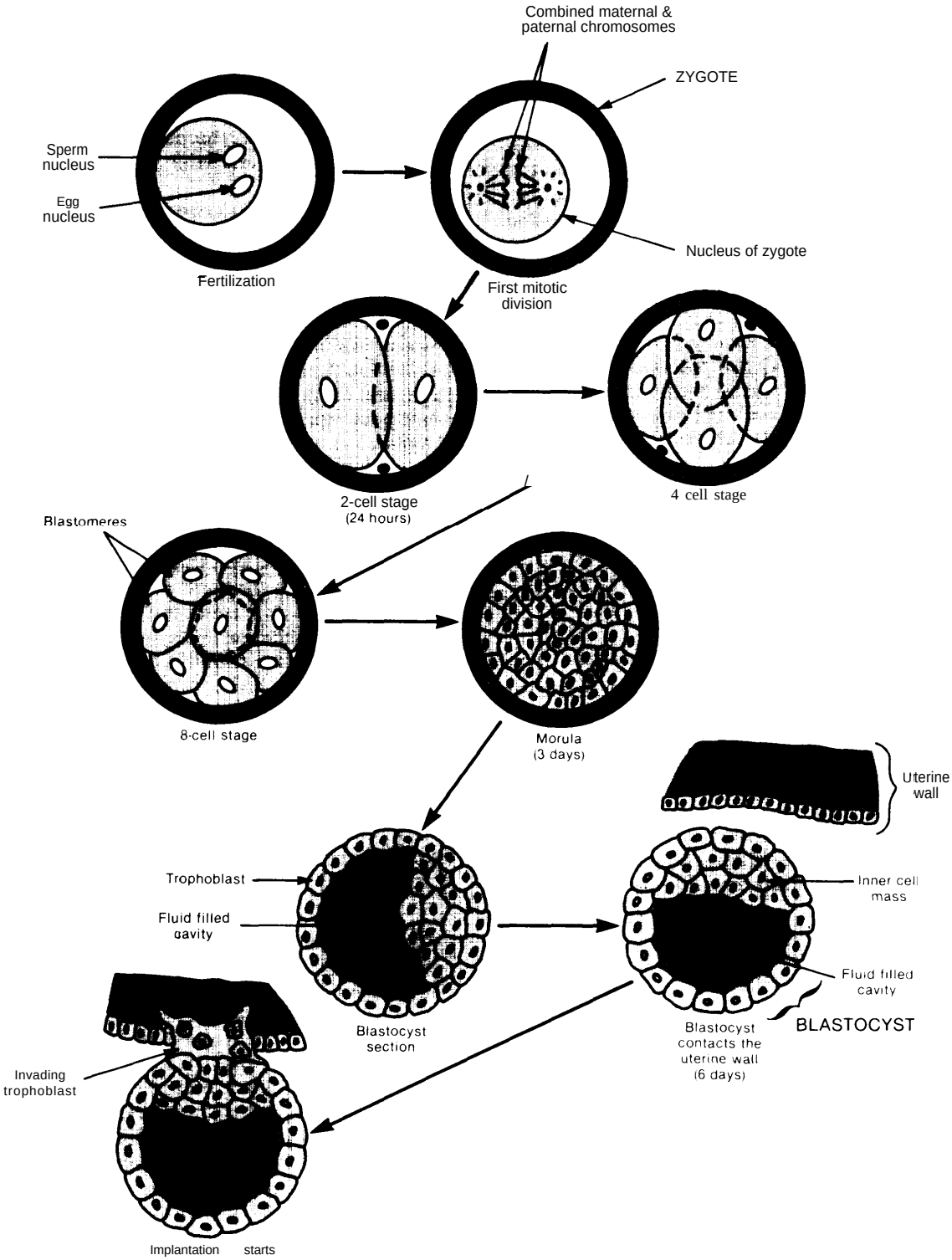
At the same time the primitive placenta is forming, the cells that will later become the embryo, and then fetus, become more distinct from those embryonic cells that develop into the supporting structures (placenta and protective membranes). By 2 weeks postfertilization the process of implantation is almost complete, and differentiation of the embryo itself is becoming more pronounced: at least two distinct classes of embryonic tissue can be identified. The third week sees the emergence of a group of cells called the primitive streak that will eventually lead to the development of the nervous system, which begins before the end of the third week after fertilization. The primitive streak is the first landmark that distinguishes the "top" from the "bottom" of the embryo.

The embryo rapidly continues to develop more defined features, including limbs, organs, ears and eyes. About 8 weeks after fertilization (7 weeks after implantation) most of the basic tissues have taken shape. It is at this point that the embryo makes the transition to a fetus, with most subsequent development taking the form of growth and specialization of organ function, rather than the formation of new organs. Highly complex systems, like the brain and nervous system, continue to develop long after the embryo has become a fetus, and even after birth.

VIABILITY

Viability is the term used to indicate that the fetus could survive outside the womb. The concept of viability played a central role in the Supreme Court **decision in Roe v. Wade, in which maternal rights with respect to abortion were decided. The point at which viability begins has been considered to be about the beginning of the third trimester of normal gestation. This is subject to change, however, with innovation and progress in postnatal care. New techniques are proving to be efficient at preserving the lives of younger and smaller premature infants, and the trend promises to continue. The effect of these changes on the medical determination of fetal viability and its relation to maternal legal rights is not at all clear.**

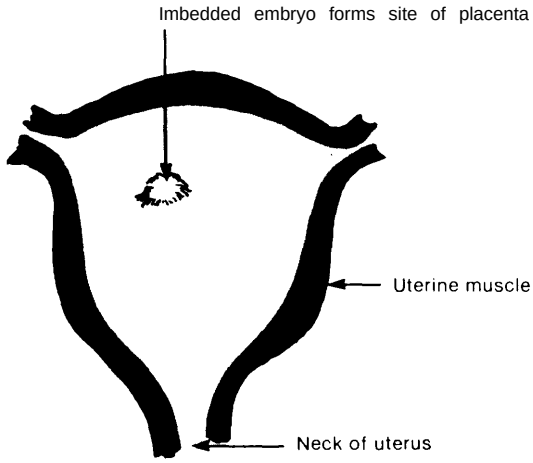
Human Fertilization and Early Embryonic Development



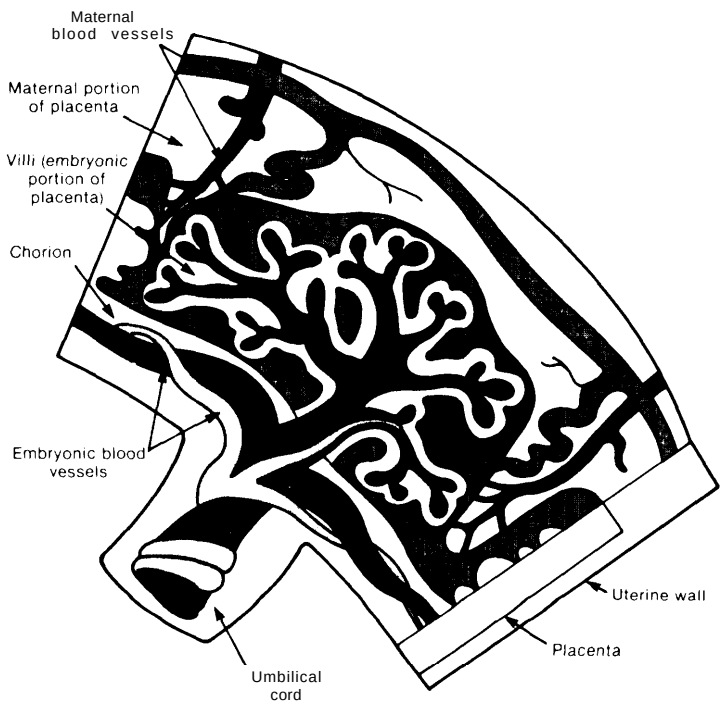
SOURCE: Office of Technology Assessment

Implantation of the Embryo in the Wall of the Uterus

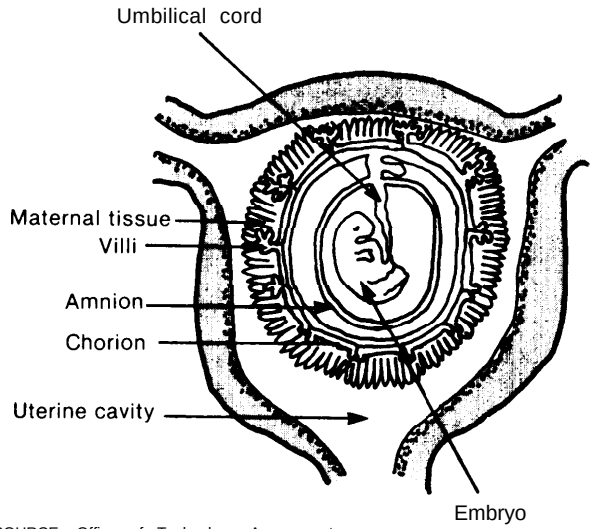
Vertical section: Human embryo, on about the 10th day, becomes embedded in the soft uterine wall. After about 2 additional weeks, the embryo will derive nourishment through a new placenta which will develop at the site of the attachment



Human Placenta

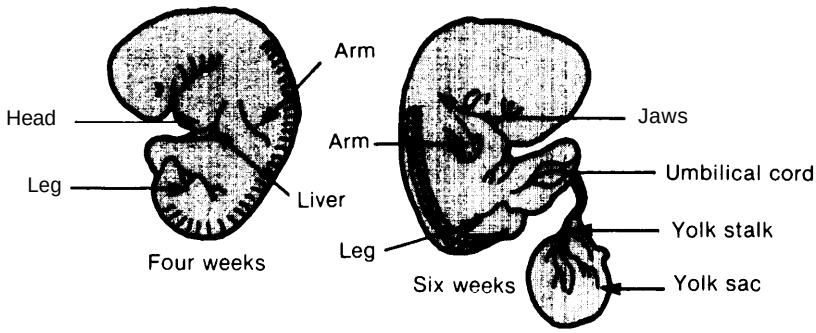


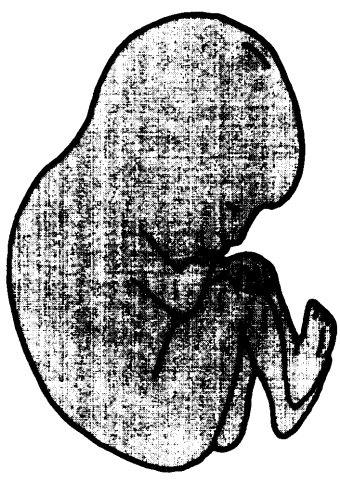
Fetal Position in the Uterus



SOURCE: Office of Technology Assessment.

Human Embryonic and Fetal Stages





15 weeks

SOURCE: Office of Technology Assessment.

Technical note 5

Hemoglobin disorders: a case study of genetic disease

Inherited hemoglobin disorders are currently the best studied and defined of all human genetic diseases. They are probably the most common single-gene diseases in the world (Weatherall and Clegg, 1981). Because of that, and because the blood- manufacturing cells in bone marrow are so accessible, hemoglobinopathies were presumed, until several years ago, to be the first candidates for human gene therapy.

Although innovative recombinant DNA technology has pinpointed the genetic defects responsible for different hemoglobin disorders, recent experiments revealing that the regulatory complexities in the manufacture of hemoglobin, and in gene regulation in general, indicate that hemoglobinopathies will not be effectively treated until these processes are better understood and can be controlled (Anderson, 1984).

NORMAL HUMAN HEMOGLOBIN

All normal human hemoglobins are composed of two pairs of identical protein chains, forming a "tetramer". Hemoglobin differs between the embryo, fetus and postnatal human because genes coding for different protein chains are activated progressively during development. Fetal hemoglobin (HbF), for example, is composed of two alpha and two gamma chains while the adult version contains two alpha and either two beta (95 percent) or, much less commonly (3 percent), two delta chains (Orkin and Nathan, 1981). **These complex regulatory changes in hemoglobin synthesis aid in transporting oxygen across** the **placenta, from mother to fetus. This is possible because embryonic and fetal hemoglobins have higher oxygen affinities than normal adult hemoglobins.**

The two major types of single-gene hemoglobin diseases are sickle cell anemia and the thalassemias.

Table 1.—Human Hemoglobins

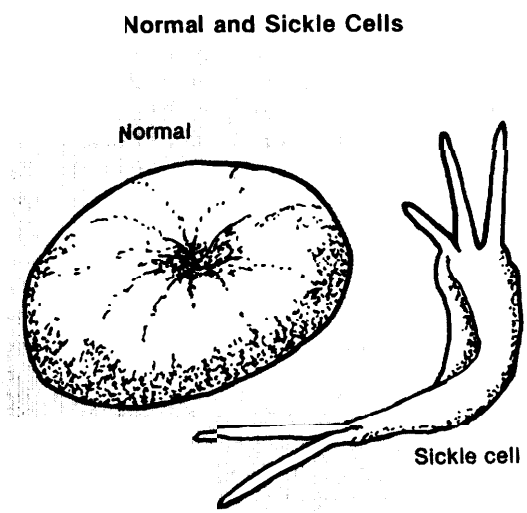
Type of hemoglobin	Chain composition	When present
Hb A	$\alpha_2\beta_2$	Adult life (~95%); small amount during fetal life
Hb F	$\alpha_2\gamma_2$	Fetal life (predominant); adult life (~1-2%)
Hb A_2	$\alpha_2\delta_2$	Adult life (~3%)
Gower-1	$\zeta_2\epsilon_2$	<12 weeks gestation
Gower-2	$\alpha_2\epsilon_2$	<12 weeks gestation
Portland	$\zeta_2\gamma_2$	<12 weeks gestation

SOURCE: Orkin, S. H. and Nathan, D. G. "The Molecular Genetics of Thalassemia" In: H. Harris, A. Hirshorn (eds.) *Advances in Human Genetics* (New York: Plenum Press, 1981) vol. 2, p. 233-288.

These genetic defects are not located on a sex chromosome and usually require two faulty copies of the gene for the disease to manifest clinically. Hundreds of thousands of people have only one faulty copy of the gene out of the allotted two, and thus are labelled heterozygous carriers of these diseases. An estimated 200,000 people with hemoglobinopathies are born annually, divided equally between sickle cell anemia and thalassemia (WHO, 1982). The thalassemias are most common in Asia, and sickle cell is most common in Africa. Among American blacks, about 8 percent carry sickle cell trait and one in 500 newborns have sickle cell disease (Stern, 1973; McKusick, 1983; Bowman, in personal communication, 1984).

Sickle cell anemia

Sickle cell anemia involves a variation in hemoglobin structure due to substitution of one nucleotide on the beta globin gene, leading in turn to a substitution of the amino acid glutamate for valine (the normal sixth amino acid on the beta globin chain) when the faulty gene is '(transcribed" and used to produce hemoglobin protein in the bone marrow cells. The hemoglobin containing the faulty beta globin chains (Hbs) is less soluble than normal hemoglobin and, under reduced-oxygen conditions, can form a crystal that distorts the red blood cells into shapes resembling



Red blood cells–normal and sickle cell

SOURCE: Office of Technology Assessment.

sickles. These misshapen red blood cells are rapidly destroyed and become lodged in capillaries, leading to partial or total blockage of blood supply to parts of the body. Pain and local-tissue damage results, especially in those organs with extensive capillary networks such as the lungs, heart, kidneys, brain, spleen and hips.

The blood of a person with two copies of the "sickling" gene consists primarily of HbS; this person is said to have "sickle cell disease" and generally has an abbreviated lifespan. The impaired circulation can lead to anemia, pain in the joints, sporadic abdominal pain, lung and spleen damage, ulcerations of the lower extremities and acute episodes such as stroke, kidney failure, and heart failure (Bowman and Goldwasser, 1975). The clinical symptoms are extremely variable, however, and some people may remain completely free of serious illness. A person who possesses one faulty and one normal copy of the beta globin gene has "sickle cell trait" and has blood containing 20 to 40 percent HbS. Such "carriers" typically exhibit little or no clinical symptoms of anemia and have a normal life expectancy.

Thalassemias

The thalassemias are characterized by decreased production of certain hemoglobin chains, There are several types of thalassemia named according to which globin chain is deficient. For example, in alpha thalassemia, little or no alpha globin is produced. The same holds true for beta thalassemia, where little or no beta globin is produced. These are the most common forms of thalassemia.

The decrease in globin production by the affected gene ranges from none at all (as in alpha^{o}- or beta^{o}-thalassemias) to somewhat less than normal (as in alpha^{+}- or beta^{+}- thalassemia). The clinical signs and symptoms are extremely variable, especially among heterozygotes, ranging from none to serious anemia. Generally, the symptoms afflicting an individual heterozygous for thalassemia are exacerbated under physical stress. Prolonged stress can exhaust the auxilliary blood production mechanisms that are already being pushed to maintain normal hemoglobin levels.

The genetic defects underlying thalassemias are as varied as the associated clinical symptoms. The impaired synthesis of globin chains could result from mutations grossly affecting the structure of a globin gene, decreased transcription of the gene, abnormal RNA processing, or defects in the activity and translation of the mature RNA (Treisman, Orkin, Maniatis, 1983).

ALPHA THALASSEMIAS

Most alpha thalassemias involve gene deletion. "Silent carriers" (those showing no clinical symptoms) have one of the four normal alpha globin genes per cell deleted. Those with alpha thalassemia "trait" have two genes deleted and usually show no anemia. "Hb H" disease is associated with the deletion of three genes (Kan, et al., 1975) and is characterized by mild to moderate anemia. Homozygous alpha thalassemia involves deletions of all four gene copies and results in severe anemia, accumulation of body fluid, and intrauterine death (Orkin, 1978).

BETA THALASSEMIAS

There are only two beta globin genes in the normal human genome. If only one copy of the gene is affected, an individual is said to be heterozygous and have beta thalassemia trait. Such heterozygotes are usually asymptomatic, except for occasional mild anemia or slight spleen enlargement. If both genes are affected, the individual is homozygous and has the disease beta thalassemia. Symptoms of the beta thalassemia disease include severe anemia, enlargement of the spleen, liver and heart, skeletal deformation, abnormal facial features, and abbreviated life span.

Other less common forms of thalassemia involve persistence of fetal hemoglobin, however, and therefore constitute models for the study of the mechanisms responsible for switching from fetal to adult hemoglobin synthesis during development. If better understood, this process might be exploited clinically as a treatment for beta thalassemia in which fetal hemoglobin synthesis could be "turned on" to compensate for deficient adult beta globin synthesis.

Other unstable hemoglobins

There are dozens of mutant types of globin protein that replace either the alpha or, more commonly, the beta chains in hemoglobin (Winslow, 1983). Many of these form unstable hemoglobins that deteriorate rapidly and cause anemia. Most are extremely rare, with the exceptions of hemoglobin SC disease and hemoglobin S-thalassemia. These two disorders are hemoglobinopathies that occur in patients who have one sickle cell gene combined with another mutant gene–for globin C in one case and for thalassemia in the other.

The unusual hemoglobinopathies vary widely in clinical severity. Most are relatively well understood. Gene therapy for most of them would involve the same steps in replacing defective genes in bone marrow cells with their normal globin gene counterpart.

Diagnosis of hemoglobinopathies

Because the symptoms of the hemoglobinopathies are very heterogeneous, a definitive diagnosis usually requires assays for abnormal hemoglobin or DNA analysis. The simplest and most common method of diagnosing sickle cell trait and anemia postnatally is through protein electrophoresis of a blood sample (see diagram). Because of the risk associated with fetal blood sampling, however, this procedure may soon be displaced in *prenatal* diagnosis by the less risky procedure of DNA analysis of cells obtained through amniocentesis or chorionic villus biopsy (see app. A).

Electrophoresis can also be used to detect most forms of thalassemia postnatally, except for the "silent carrier" form of alpha thalassemia which may now be diagnosed using restriction endonuclease DNA analysis (Embury, et al., 1979). Prenatal detection of homozygous beta thalassemia has been possible since 1974 through quantitation of the amount of beta globin manufactured by a fetal blood sample (Kan, 1977; Alter, 1979). Prenatal diagnosis of certain forms of beta thalassemia is also possible using DNA analysis (Alter, 1981; Antonarakis, 1982; Boehm, 1983; Connor, 1983; Estein, 1983; Hodgkinson, 1984; Orkin, 1982, 1983; Pirastu, 1983).

Treatment of Hemoglobinopathies

Currently, clinical treatment of hemoglobinopathies is limited largely to treatment of infections, mitigation of the associated symptoms (e.g., pain in the joints), and organ-specific therapy (Dean and Schecter, 1978). There is no effective long-term treatment for sickle cell anemia, and the two treatments available for thalassemia are only partially effective, with undesirable side effects (Adamson, 1984). The first treatment involves repeated transfusions with normal red blood cells can alleviate some of the symptoms, but eventually leads to toxic iron overload. The second treatment, bone marrow transplants, or the transfer of healthy bone marrow from a relative into the patient, has been used successfully to treat homozygous beta thalassemia. This carries a high risk of failure, however, and the possibility of an immune reaction of the patient against the transplanted marrow.

It could be argued that prenatal diagnosis obviates the need for postnatal treatment. However, there will always be children born with hemoglobinopathies and other genetic diseases because: 1) parents often do not realize that they are carriers until they have had an affected child; 2) parents who know they are carriers may chose to take the risk of their child having a genetic disease; 3) prenatal diagnosis is often unacceptable for moral, ethical, religious, or personal reasons; and, 4) genetic mutation is constantly reintroducing defective genes.

Several alternative treatments are currently being developed experimentally that may be divided into three categories: 1) drug therapy, 2) gene therapy, and 3) bone marrow transplant (Desnick, 1981). To date, no form of gene therapy and only a handful of the drug therapies have progressed to the point of clinical trials, and bone marrow transplant appears to be of possible use for only a small percentage of patients.

DRUG THERAPY

Two types of drugs are currently being developed to treat hemoglobinopathies. One type is designed "turn on" the synthesis of fetal hemoglobins to compensate for the faulty or insufficiently produced adult hemoglobins. Some of these drugs are already being tested clinically (Dover, 1983, 1984). The second type is meant to suppress the polymerization or gelling of the sickle hemoglobin molecule that distorts the red blood cells. Some of these drugs have also been tested clinically (Bookchin, 1976; Dean, 1978; Lubin, 1975; Nigen, 1974).

GENE THERAPY

Treatment of hemoglobinopathies through gene therapy, or the insertion of normal globin genes into the embryo (germ line) or into bone marrow (somatic) that is then implanted, is still entirely in the experimental stage in animals. The success rate of in vitro germ-line transplants is still disappointingly low (see app. B). The lack of animal models for hemoglobinopathies has effectively hindered both germ-line and somatic-cell experiments. Recently, however, a model for beta thalassemia was developed in the mouse (Skew', et al., 1983). Even given such models, however, the researcher is faced with the task of having the gene express at all, at adequate levels, at the right time, and in the right tissues in the whole animal.

BONE MARROW TRANSPLANT

Gene transplant for hemoglobinopathies attempts to take advantage of the relative accessibility of human bone marrow cells, where hemoglobin is produced. Bone marrow is removed from a donor who produces normal hemoglobin, who has been matched for tissue compatibility with the recipient patient who suffers from a disorder of hemoglobin. The donor patient then receives radiation treatment sufficient to destroy the cells of his own bone marrow. Once accomplished, the patient receives the transplants of the donor's bone marrow. The recipient is then treated with drugs to

suppress his or her immune reaction against the donated cells (but this also affects general body defenses). If not rejected by the host, the transplanted bone marrow begins to manufacture normal hemoglobin. The procedure is quite stressful to the patient, relatively risky, and not all patients can be matched with compatible donors.

Appendixes

Appendix A

Diagnostic Technologies for Genetic Diseases

Diagnosing genetic diseases requires a partnership of two types of technologies: those for sampling bodily tissues and fluids, and those for analyzing such samples obtained before and after birth. Although fetal imaging is not tissue analysis in the strict sense, it will he discussed here as a technology useful both in conjunction with prenatal tissue sampling and by itself to view gross congenital malformations in utero. This section reviews the major imaging, sampling, and analysis techniques, and comments on their current and potential use as clinical tools. It applies only to prenatal and early postnatal diagnosis.

Often, the only 'treatment" available for a fetus diagnosed as having genetic disease is the elective termination of pregnancy. All the stigma, emotion, and ethical controversy attached to this possible recourse can be-and has been—transferred to the diagnostic techniques themselves, and exacerbated by those techniques having been inapplicable until well into the 2nd trimester of pregnancy. With the advent of techniques minimizing risk to the fetus and allowing diagnosis within the first trimester, prenatal diagnosis may become more accepted.

Controversy also attends the use of postnatal diagnostic techniques. Genetic testing for certain disorders among high-risk populations—such as the screening for sickle cell trait among American blacks in the 1960s--have been said to stigmatize and demean individuals in those populations. Recent advances in diagnosing genetic disorders whose clinical symptoms often do not surface until adulthood—such as Huntington disease or familial hypercholesterolemia (a predisposition for arterial hardening which causes early heart attacks)—have raised further questions: Would a potential employer or insurance company have a right to this information? (see app. B). Propelled by rapidly advancing rDNA technologies, the expanding use of these diagnostic techniques will soon necessitate an answer to these ethical and political questions.

Fetal imaging

Fetal imaging involves obtaining a visual image of the fetus, either by means of special electronic techniques, or by using fiberoptic. Ultrasound and fetoscopy are the two major types of imaging. They can be used in and of themselves, as well as being partnered with techniques for tissue sampling (see below).

ULTRASOUND

Ultrasound is commonly used in determining fetal age and defining large anatomic structures. It involves high-frequency sound waves, undetectable to the human ear, that are directed toward the uterus. A fetal image is created from the differential reflection of the sound waves bouncing off diverse fetal tissues. Various gross congenital malformations may be detected using ultrasound, including hydrocephalus (excess fluid of the brain), anencephaly (absence of all or most of the cerebral hemispheres), absent or stunted limbs, and some defects of the heart and kidney (Hobbins, Venus and Mahoney, 1981). For purposes of tissue sampling, it is generally used in conjunction with amniocentesis (see below). There is currently little evidence of risk to the fetus from the small doses of ultrasound needed for in utero visualization. However, cautioning against routine screening, the NIH Consensus Development Conference on Diagnostic Ultrasound Imaging in Pregnancy stated in its findings, '(Lack of risk has been assumed because no adverse effects have been demonstrated clearly in humans. However, other evidence dictates that a hypothetical risk must be presumed with ultrasound. Likewise, the efficacy of many uses of ultrasound in improving the management and outcome of pregnancy also has been assumed rather than demonstrated, especially its value as a routine screening procedure . . . .Ultrasound examinations performed solely to satisfy the family's desire to know the fetal sex, to view the fetus, or to obtain a picture of the fetus should be discouraged. In addition, visualization of the fetus solely for educational or commercial demonstrations without medical benefit to the patient should not be performed" (Office of Medical Application of Research, 1984).

FETOSCOPY

Fetoscopy entails the insertion of a thin fiberoptic scope through the abdomen into the uterus. The procedure usually is done around the 18th week of gestation. It permits a well-defined narrow-angle view of

isolated parts of the fetus, and thus is used for fetal surgery as well as imaging. Fetoscopy, however, primarily is used to obtain fetal tissue samples (see below).

Technologies for fetal tissue sampling

The three major fetal tissue sampling technologies in use today are: fetoscopy, amniocentesis, and chorionic villus biopsy. Fetoscopy is infrequently used because it is relatively risky and difficult to perform. Amniocentesis is relatively safe for both the fetus and mother, and is widely used today. Chorionic villus biopsy still is largely in the developmental stage in the United States, but has certain advantages that may lead to its widespread use in the near future.

FETOSCOPY

Direct tissue sampling via fetoscopy makes use of the fiberoptic scope—inserted into the uterus for fetal imaging purposes—to remove blood and skin samples, A needle or forceps, guided through the fetoscope by ultrasound, accomplishes this purpose, Various hemoglobinopathies, muscular dystrophy, and hemophilia can all be diagnosed using fetal blood samples (Hobbins, Venus and Mahoney, 1981). However, with the advent of sensitive DNA analysis techniques in the late 1970s, diagnosis of hemoglobinopathies such as sickle cell disease and thalassemia can now be obtained through the less risky procedure of amniocentesis (see below). Fetoscopy, never widely practiced, carries a 3-to-6 percent risk of fetal death over and above the natural losses from spontaneous abortion and miscarriage (Alter, et al., 1981; Rocker and Laurence, 1981), and is routinely performed at only a few medical centers.

AMNIOCENTESIS

Amniocentesis involves sampling fetal cells and other substances present in the amniotic fluid. This is accomplished via a needle inserted through the abdominal wall, through the wall of the uterus, and into the fluid-filled space that surrounds the fetus. Amniocentesis normally is done using ultrasound to direct the needle.

First used in the late 1960's amniocentesis is now routinely employed with chromosome analysis techniques to detect abnormalities such as Down's syndrome, neural tube defects (through testing of the amniotic fluid), enzyme deficiencies (as in Fabry disease and Lesch-Nyhan syndrome), and hemoglobinopathies (through testing of cultured fetal cells). Amniocentesis is widely available, has a low associated risk of fetal death (less than 0.5 percent), and is 99.4 percent accurate (NICHD, 1976).

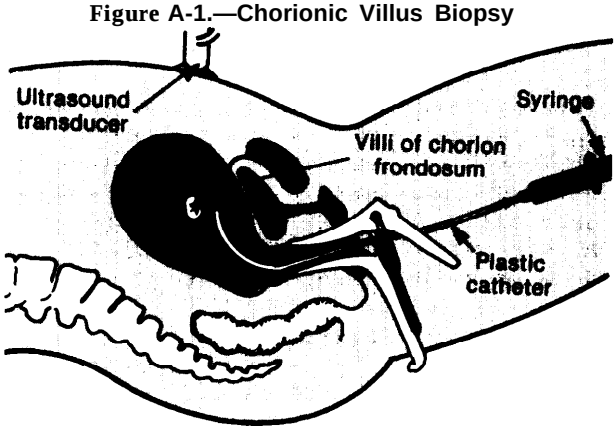
Theoretically, amniocentesis could be performed early in the pregnancy since DNA analysis allows detection of the defect in any cell, regardless of tissue type or stage of fetal development. However, not enough fetal cells are available in the amniotic fluid early on, and thus, amniocentesis is usually performed no earlier than between the 16th and 19th weeks of pregnancy, Additionally, the cells from the fluid must often be cultured for 1 to 5 weeks to yield a large enough tissue sample for analysis, further delaying diagnosis. Thus, with amniocentesis the abortion of an affected fetus, if elected, must be performed well into the second trimester when the results of the analysis become available. Such a delay increases the risk of complications associated with abortion including trauma, sepsis, and hemorrhaging (Brash, 1978). Abortion that late into a pregnancy also can carry a considerably increased risk of psychological and physical stress to the parents, and is generally less acceptable to parents and the community. The further development of methods to analyze *uncultured* amniotic fluid cells continues to greatly speed diagnosis-e. g., a new technique for examining uncultured cells with an electron microscope can diagnose a glycogen storage disease 3 to 6 days after amniocentesis (Hug, et al., 1984). One of the consequences of this increasing effectiveness in neonatal and premature care is that the age at which viability is reached (see Technical Note 4) is constantly being pushed back, mandating earlier parental decisions vis a vis the course of the pregnancy.

CHORIONIC VILLUS BIOPSY

Chorionic villus biopsy (CVB) is a relatively new technique for the sampling of fetal tissue that can be performed as early as the 8th to 10th weeks of pregnancy. It entails taking a sample of the fronds of tissue, or villi, that root the fetal placenta to the uterus. Ultrasound is used to guide a catheter into the woman's uterus to the villi, a tiny bit of which is then suctioned off using an attached syringe (see fig. A-l). The fetal tissue is then separated from maternal tissue and subjected to biochemical or chromosomal analyses that take an average of one week to yield a diagnosis. In contrast to amniocentesis, extensive culturing of the tissue is not necessary since a sufficient quantity of DNA is obtained in the tissue sample. CVB can be performed weeks, even months, earlier than either amniocentesis or fetoscopy.

CVB was first used in China (Department of Obstetrics and Gynecology, Anshan, 1975), the U.S.S.R. (Kazy, Rozovsky and Bakarev, 1982), and Norway. It

Figure A-1.—Chorionic Villus Biopsy



SOURCE: Product News, "Chorionic Villus Biopsy," *Outlook*, January 19S4, p.8. Carolyn Brooks, artist,

has been considered ethically acceptable in England and used since mid-1981 for prenatal diagnosis of certain "high-risk" disorders such as hemoglobinopathies (Old, et al., 1982), and fetal sexing of pregnancies at risk of sex-linked diseases (Gosden, et al., 1982), mainly Duchenne muscular dystrophy. It is used less often there to diagnose the more common Down's syndrome. Unlike amniocentesis, however, CVB cannot **be used to detect noncellular substances in the amniotic fluid, like alpha fetoprotein, whose presence indicates a high risk of neural tube defects.**

According to the World Health Organization's registry, CVBs have been performed in the United States since mid-1983. By November of that year, a projected 12 percent associated fetal loss rate was reported after only 240 CVBs had been performed in the U.S. and Europe (Ward, as cited in Jackson newsletter, 1983). Because of this, several researchers (Lippman, 1984; Hecht, Hecht, Bixenman, 1984) contend CVBs are risky and should be used with caution. Current clinical data from approximately 2900 CVBs performed to date in these same countries seems to indicate that the observed fetal loss is 4.2 percent (Jackson, 1984 newsletter). This, however, includes an unquantified number of spontaneous abortions and miscarriages that are a danger to any normal pregnancy. In four similar series of ultrasound observations on ultrasound pregnancies and control groups, the observed fetal loss rate is in the neighborhood of 2 percent. Extrapolating from that, it may be reasonable to assume that the risk of fetal loss that might be associated with CVB is 2 to 3 percent. Any fetal loss rate, however, is highly dependent on the expertise of the laboratory involved, and there are laboratories with a reported O percent observed fetal loss (Jackson, 1984 newsletter). Although generally higher than the 0.5 percent loss rate associated with amniocentesis, **much of the discrepancy may be due to the time when the procedures are performed. There is a higher spontaneous abortion rate in the first trimester, when CVBs are done, than in the second trimester, when amniocentesis is done. Some even predict that CVB will replace amniocentesis within a few years (Product News, 1984).**

Current clinical applications of chorionic villus biopsy include at least three groups in England, with four or five more testing it for clinical use in that country (Dr. Robert Williamson, Ph. D., personal communication, 2-16-84). Other countries include Italy (over 200 cases of Down's syndrome have been diagnosed by a Milan group) and France (to diagnose hemoglobinopathies; Dr. Robert Williamson, Ph. D., personal communication, 2-16-84).

Tissue and fluid analysis

Genetic diseases were first detected through their characteristic behavioral or physical traits. This approach, combined with family histories, still is important to the diagnosis of many genetic diseases, especially those for which the underlying biochemical and genetic defects are not known. However, behavioral and physical examination is generally not applicable to prenatal diagnosis, or on the cutting edge of diagnostic technologies, and will not be discussed here.

Many genetic diseases have surfaced in recent years whose physical and behavioral manifestations are not readily apparent, are progressive, or take several years to emerge. Many such diseases are detectable through biochemical assays for characteristic imbalances or abnormalities of certain body substances.

A small but growing number of diseases may now be diagnosed through direct analysis of the genetic material. This was once only possible for gross chromosomal abnormalities involving the absence or duplication of entire chromosomes, but recent advances in molecular genetic technology have made possible detection of minute defects within the chromosomes. Such genetic analysis can allow diagnosis of the disease before the biochemical defect is detectable, especially prenatally, and before it becomes clinically apparent, making possible early treatment and sometimes even prevention.

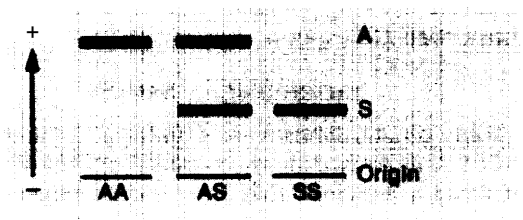
BIOCHEMICAL ASSAYS

Many genetic diseases are manifested as biochemical imbalances caused by the reduced or absent activity of certain enzymes that help manufacture a given chemical, or convert it into another useful product. Such enzyme deficiencies underlie a spectrum of dis-

orders ranging from albinism to hemolytic anemia to some immunodeficiency diseases. X-ray, urine analysis (for excretion of abnormal amounts of certain accumulating precursors) and physical or mental examinations are often used for preliminary detection. However, enzyme assays of the blood or other tissues are generally necessary to make a definitive diagnosis of such diseases. Tay-Sachs disease (TSD), for example, is a metabolic disorder primarily affecting Jews of Eastern European descent (1:3,000 US.) (Stanbury, et al., 1983) caused by the lack of an enzyme, Hexoseaminidase A, that results in the accumulation of lipids in the brain. TSD is characterized by progressive neurological degeneration including dementia, paralysis and blindness. Diagnosis routinely involves enzyme assays of cultured amniotic fluid cells prenatally and of the blood serum postnatally.

Lack of activity of an enzyme or other substance is sometimes due not to a quantitative lack of the substance, but to a structural defect that prevents it from functioning properly. Electrophoresis is a method of distinguishing such variants through the different speeds at which they migrate in an electrical field according to their total net charge—a characteristic that may vary with molecular structure. For example, electrophoresis can reveal the absence of any one of the three major classes of immunoglobulins—as well as variations within any one class—that characterize certain immune deficiency diseases. It also can detect and distinguish between both heterozygotes (i.e., sickle cell trait) and homozygotes (i.e., sickle cell disease) for the sickle cell gene (see fig. A-2). Protein electrophoresis is a relatively inexpensive and expedient technique, and is commonly used for postnatal detection of hemoglobinopathies. Because of the relatively high risk involved in obtaining fetal blood samples (see Technologies for Fetal Tissue Sampling, this appendix), electrophoresis is less commonly used for prenatal diagnosis. There are some cases in which electrophoresis is insufficient to distinguish particular genotypes, and therefore is frequently combined with a volubility test. In most cases, these two tests will suffice to identify a hemoglobin disorder.

Figure A-2



SOURCE: Bowman, J. E. and Goldwasser, E. (1975) Sickle Cell Fundamentals, The University of Chicago.

DIRECT ANALYSIS OF DNA

Cytogenetics: Visualization of Chromosomes.—Cytogenetics is the examination of chromosomes under a microscope in order to detect gross changes in chromosomal structure. One of the first clinical applications of this technique was the detection of Down syndrome (Lejeune, 1959), in which the cell carries an entire extra chromosome 21. Although many reports on other numerical chromosomal aberrations followed, researchers were often unable to identify which chromosome was involved. Characterization of banding patterns on particular chromosomes in the late 1960s and early 1970s allowed the identification of specific chromosome pairs as well as parts of each chromosome (Hirschhorn, 1981). An array of chromosomal deletions, duplications and translocations, and the corresponding syndromes, have since been identified (Borgoankar, 1980). Down syndrome and other major numerical and structural chromosomal defects afflict about 1 in 160 live-born infants (Hook and Hamerton, 1977). Such chromosomal defects—including abnormalities of the sex chromosomes **such as Klinefelter syndrome in which the male possesses an extra X chromosome, causing sterility and feminization—are now routinely diagnosed both before and after birth using cytogenetics.**

Genetic Markers.--The rapid progress in recombinant DNA techniques since the mid-1970s has made possible detailed analysis of particular genes on the chromosomes, and the characterization of minute genetic defects that are not detectable through examination of gross chromosomal structure. In recent years, the defective genes underlying various other heritable diseases have been identified—including Lesch-Nyhan syndrome (Jolly, et al., 1982, Brennand, et al., 1982) familial hypercholesterolemia (Bishop, 1983), and phenylketonuria (Woo, et al., **1983). Within the past 2 years, genetic "markers" (though not the actual genetic defect) have been discovered for two important genetic disorders: Duchenne muscular dystrophy (Murray, et al., 1982) and Huntington disease (Gusella, et al., 1983).**

The discovery of identifiable markers for genetic defects opens up the possibility of using them to aid in diagnosis. Such markers might be useful as prenatal tests, for postnatal identification of risk, or perhaps even for genetic screening. For example, phenylketonuria (PKU), an inherited enzyme deficiency that, if untreated, can cause severe mental retardation, could not be detected before birth until recently (although biochemical tests were available for postnatal screening). The recent discovery of a genetic "probe)" or

short stretch of DNA specific to the defective gene, has made possible not only prenatal detection, but also carrier identification (Woo, et al., 1983). This means that parents at risk of having a child affected by PKU can be identified. This opens up the technological prospect of parental carrier screening to supplement or replace routine newborn screening.

Direct analysis of DNA is being used now in the diagnosis of several other diseases as well, including some disorders of hemoglobin and one form of dwarfism (Antonarakis, et al., 1982). Direct binding of DNA probes to patient DNA could theoretically be developed for any disease caused by a single gene.

Genetic markers can be of several types. Some depend on the presence or absence of specific biochemical activities. Others depend on the differences in how DNA is cut by enzymes specific to certain nucleotide sequences in combination with DNA probes—specific detectable stretches of DNA constructed in the laboratory that bind directly to either the gene causing the disease or a piece of DNA so close to the disease-causing gene that it can be used as an indicator for the defective gene. The techniques are briefly described below.

Restriction Enzymes. -Restriction enzymes are proteins that are found in bacteria that cut DNA at specific sequences. Human DNA is composed of roughly 3 billion pairs of nucleotides (see Technical Note 1); restriction enzymes look for stretches of 4 to 12 nucleotides that are arranged in a particular order, and cut the DNA at a site either in the middle of the sequence or near to it. When the DNA from human cells is so treated, DNA fragments of many lengths are generated. The DATA from any one individual will have a specific pattern because-the sequence recognized by a particular enzyme will occur in characteristic places in that person's DNA.

People generally have very similar patterns of DNA fragmentation when their DNA is treated with restriction enzymes, and so most enzymes have not yet been shown to be useful for diagnosis. Some enzymes, however, generate differences that correlate with disease. The DNA coding for sickle cell disease, for example, is cut by an enzyme that does not cut the normal gene. Therefore, when this enzyme is used on DNA from a patient, the one DNA fragment found in normal individuals is cut into two smaller pieces. These pieces can be seen using standard laboratory methods, and the technique has been used to detect both sickle cell disease and sickle cell trait (Orkin, et al., 1982).

In most cases, the differences between the normal and the abnormal gene will not be so easily identified: the disease-causing mutation will not occur where restriction enzymes are known to cut. In this case, one can sometimes identify people who might carry the abnormal gene by identifying differences in a piece of DNA close to the gene causing the disease. This technique depends on using restriction enzymes indirectly, rather than directly, and is correspondingly less precise.

People show characteristic variations in how their DNA is cut by certain restriction enzymes, just as they have specific blood groups. These variations usually are not significant in and of themselves. The place along the DNA that is responsible for the variations can be located. The utility of such variations comes when, by chance, a particular pattern is caused by differences close to a disease-causing gene. When this occurs, it is often possible to track the abnormal gene by following the restriction fragment pattern. This technique of establishing "guilt by association" is called linkage analysis (denoting a physical genetic linking between a trait of interest and an identifiable marker) and has permitted tracing of the Huntington disease gene, (Gusella, et al., 1983) the Duchenne muscular dystrophy gene, and genes underlying several hemoglobin disorders (Boehm, et al., 1983). Because the technique does not require any special knowledge about which gene causes a disease, or the biochemical lesion responsible, restriction fragment analysis may be used to diagnose diseases whose molecular mechanisms are not yet known, such as cystic fibrosis. A major disadvantage of the technique is that the restriction enzyme pattern usually varies from family to family, and many members of each family must be tested before genetic detection within any one family is practical.

The technique is analogous to searching for passengers of a downed plane. In thousands of miles of mountainous territory, the wreckage of an aircraft is found by tracing its radio distress signal. This does not give much information about the condition of the crew or the circumstances of the crash, but it does permit restriction of the search to a smaller area, and increases the probability of finding the passengers. The crash site itself may provide some clues about the cause of the mishap and where to look for survivors. In this analogy, the radio signal is like a linked genetic marker, while the defective gene is like the crash site.

DNA Probes.--Gene probes are short stretches of DNA that bind to a specific DNA sequence. Through cloning, many identical copies of a probe can be made. Probes are usually made out of DNA that has been specially labelled with either a radioactive or chemical tag that allows the probe to be used to detect specific DNA sequences, employing standard laboratory met hods.

Probes are often used in combination with restriction enzymes. First the DNA is chopped into manageable sizes by restriction enzymes, and then a probe

is bound to the DNA. In the instance of the sickle cell gene mentioned above, for example, the probe corresponds and binds to an abnormal variation of the hemoglobin gene that causes the disease, allowing its detection.

Probes come in different sizes. Most sequences used as probes are fairly long, composed of many copies of a single ordered sequence of hundreds or thousands of nucleotides. These are usually made using bacterial clones of a gene or DNA fragment. Some short probes, called oligonucleotide probes ("oligo-" means few), can be chemically manufactured in the laboratory. These small highly specific probes can, under carefully controlled conditions, detect the difference between genes that differ only in a single nucleotide in their sequences. This property has been used to detect sickle cell disease (Wallace, et al., 1981; Orkin, 1982; Conner, et al., 1983), and some thalassemias. (Orkin, et al., 1983; Pirastu, et al., 1983) An oligonucleotide probe has also been developed for another genetic disease, alpha-1-antitrypsin deficiency (an inherited deficiency of a blood protein that can lead to lung and liver disease), making prenatal diagnosis possible (Kidd, et al., 1984).

The power of the new diagnostic techniques can be imagined by noting that they can detect differences of a single letter in a book composed of three billion letters. If each gene is a paragraph, then only a few paragraphs in a long monograph have been investigated using the new techniques; more of the text will be tested over the next few decades, and the meaning of the book may thus slowly become clearer.

Appendix B

Privacy and Control of Genetic Patient Data

introduction

Some of the same recombinant DNA technology that makes human gene therapy possible will also facilitate the identification of many more individuals with genetic diseases than earlier techniques allowed. This new technology should result in a dramatic increase in the amount of genetic patient data that can be collected, much of which has never been available before.[1] However, the ability to gather potentially large amounts of new genetic data about individuals raises questions about rights of privacy regarding that information, as well as the ability of others to have access to it.

WHAT ARE GENETIC PATIENT DATA?

Genetic patient data refer to information collected about an individual relating to his or her genetic constitution. Information of this sort can include a large number of individual traits, ranging from eye color or blood type to predispositions to or presence of various diseases. Since genes determine many personal characteristics, genetic data may reveal important facts about an individual's physical and intellectual status or potential. One's genetic complement is an involuntary endowment, since the genes are passed on from parents, and genetic characteristics are not generally subject to change.

Policies on access to genetic patient data must balance the benefits deriving from disclosure against the need to preserve individual privacy. The benefits to public health and other priorities often determine that medical information be disclosed. Examples of situations in which medical information is used for public good or prevention of harm include reporting child abuse or other criminal conduct, notifying State officials about the presence of communicable disease that might endanger public health, and use of disease statistics in planning priorities for biomedical research. Patients might be harmed, however, as a consequence of disclosing their genetic data. They might be socially stigmatized, have difficulty finding a mate, encounter barriers to obtaining life and health insurance, or be discriminated against when seeking employment.

Genetic patient data are different from other types of disease- related medical information, in the following ways:

- In contrast to communicable diseases, the public at large is not at risk of contracting genetic disease, since it can be transmitted only to progeny. Because of the genetic transmission of the disease, information about close relatives may reveal information about oneself, and vice versa. Closely related individuals can benefit from this information.
- Because some genetic diseases, such as Huntington disease, colonic polyposis, or polycystic kidney disease, may not be expressed until middle or old age, genetic information in some cases provides a look into the future health of an individual.
- Because of the emotional concern of the patient when learning about a genetic disease in their family against which he/she has no defense.
- Future generations may inherit the disease, and therefore have an interest in it.

Those potentially interested in genetic patient data include the patient, his or her family, insurance companies, employers, health care providers, and the Federal Government.

HOW ARE GENETIC PATIENT DATA COLLECTED?

Genetic patient data are collected about individuals in many ways, but the bulk of specific information on genetic traits derives from two main sources: family histories and genetic tests.[2]

A family history can be relatively easy to collect, and most genetic patient data available to physicians are of this type. A family history is usually obtained by asking the patient questions about the presence of diseases in his or her family that are known to be inherited. Histories can often be supplemented by inquiry among other family members. The importance of genetic factors varies between diseases. Recent data on Alzheimer disease indicate that a significant fraction, at least one-third of cases may be genetic (Breitner, 1984; Folstein, 1981; McKusick, 1983), while other diseases, such as PKU, are always due to genetic defects. Variation in the genetic component among different diseases and even among diseases of the same

[1] The number of cloned human genes is an index of this increase in potential genetic patient data The number of cloned human genes reported at the Gene Mapping Meetings has risen from 22 in 1982 to 132 in 1984 (Skolnick, et al., 1984)

[2] These include a variety of biochemical and genetic tests See app A for further information on genetic testing techniques.

type can be due to several factors, discussed in the overview, such as:

- incomplete penetrance,
- variable expression,
- environmental factors,
- different patterns of inheritance: dominant, recessive, or sex-linked,
- multigene traits, and
- multifactorial traits.

As a result of these factors, genetic patient data collected from family histories can alert individuals to personal health risks and statistical likelihoods, but it generally cannot predict with certainty whether an individual with a family history of cardiovascular disease or cancer, for example, will actually develop those disorders.

With reliable genetic tests, it is sometimes possible to determine the presence of genes that can cause disease, permitting more accurate determination of the probability of expressing symptoms. Genetic testing may be performed as a result of information obtained in the family history, or can, in some cases, be initiated to screen for diseases common in a more general population to which the patient belongs.

Genetic patient data are collected in several different contexts. Family histories are recorded when an individual first visits a physician, and generally when a person buys individual life insurance. Genetic testing is often performed in the context of making personal, medical, or reproductive decisions, and such tests are performed at different times for different reasons (Rowley, 1984). Carrier screening can identify individuals who carry one copy of a deleterious gene so that they may be made aware of the risks of having a child with a genetic disease and make a clearly informed decision about having children. Carrier screening has been performed on groups at high risk of carrying certain genes, such as Blacks and Mediterranean populations, who may have hemoglobin disorders, or Eastern European Jews who may carry Tay-Sachs disease.[3]

Prenatal screening is performed to identify possible genetic defects in the fetus and allow parents to decide whether it should be brought to term or if it might require special care when born (see app. A). Prenatal screening is indicated in several situations, including when the mother is 35 years or older, if a previous child were born with a genetic defect, or if both parents are known carriers of a gene which can be detected by such screening (Milunsky, 1980), Screening at birth can identify newborns who require special care, such as PKU newborns who need a special diet, low in phenylalanine. For this reason, newborn screening for PKU is required by most States.

Genetic screening raises many medical, ethical, legal, and economic questions, such as: 1) Can family members crucial for testing be legally coerced to participate in linkage studies (see ch. 1), 2) Should a person of any age have the right to be tested and informed of test results?, 3) Should spouses or parents be permitted to know this information?, 4) Does a child have the right to genetic information held by his parents?, 5) Should physicians inform at-risk individuals of the availability of testing?, and 6) Can the release of information from genetic testing be withheld in employment and health insurance questionnaires? (Kurlan, 1983). One of the most difficult issues is the use of abortion to prevent genetic disease. Other questions include whether the benefits of genetic screening exceed the costs of the procedure, and if so, whether newborn screening should be made mandatory (President's Commission, 1983). The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research enunciated five principles for genetic screening with the following recommendations:

1. Confidentiality. "Genetic information should not be given to unrelated third parties . . . ;"
2. Autonomy. "Mandatory genetic screening programs are only justified when voluntary testing proves inadequate to prevent serious harm to the defenseless, such as children, that could be avoided were screening performed;"
3. Knowledge. "Decisions regarding the release of incidental findings (e.g., nonpaternity) or sensitive findings (e.g., diagnosis of an XY female) should begin with the presumption in favor of disclosure . . . ;"
4. Well-being. "Screening programs should not be undertaken until the test has first demonstrated its value in well-conducted, large-scale pilot studies . . . .A full range of prescreening and followup services for the population to be screened should be available before a program is introduced;" and
5. Equity. "Access to screening may take account of the incidence of genetic disease in various racial or ethnic groups within the population without violating the principles of equity, justice, and fairness. "

This paper will not discuss further the issues related to the collection of genetic patient data; rather, it will address issues which arise after the data is collected.

[3]Screening for most genetic disorders is performed on a voluntary basis, although most States require screening of newborns for PKU and some other disorders Five States require such testing under all circumstances, 30 permit denial on the basis of religious convictions, and 9 others permit some other bases for refusal, PKU screening not required in three States (Andrews, 1984d) Some other mandatory screening laws, particularly those that involve screening of adults for potential carrier status, have been repealed because of claims made by the affected groups that they were being singled out and discriminated against (Rowley, 1984)

WHY ARE GENETIC PATIENT DATA IMPORTANT?

Genetic patient data can play an important role in the life of an individual, affecting such diverse areas as:

- **choice of spouse;**
- **psychological health**
- **reproductive decisions, such as**
  -decisions to have children
  -decisions to undergo prenatal screening, and
  -decisions to terminate pregnancy;
- **decisions about personal health risks affected by diet, smoking, and health habits;**
- **decisions about the personal health risks connected with certain jobs; and**
- **decisions concerning financial, insurance, and retirement plans.**

These are among the most personal decisions that an individual makes, and it is therefore important that their privacy be ensured. However, as mentioned above, there are others besides the individual who have an interest in genetic patient data, and their interests must also be considered.

Genetic patient data may also be significant because they have the potential for being misunderstood or misinterpreted by the public. Earlier genetic screening programs to identify carriers of sickle cell disease caused some individuals to be stigmatized because they and others did not understand the difference between the carrier state and the disease state. Some of these individuals were mistakenly treated as 'sickly' children or discriminated against in employment or insurance coverage (Rowley, 1984; President's Commission, 1983). This and other examples highlight the need for greater understanding of genetic conditions before using genetic patient data to direct social policy. As more is discovered about the genetic basis of certain diseases, such as alcoholism, schizophrenia, or complex traits such as intelligence, issues of individual privacy relating to genetic patient data may become even more important than they are today.

Privacy and access

In any discussion of the privacy of health records it is important to consider the tradeoffs between an individual's right to privacy and others' interests in having access to the same information. Privacy and access are two sides of the same coin, and to preserve an individual's right to privacy is to deny others that access. If all genetic patient data were made completely private, society would forego the potential benefits accruing from availability of that information, such as planning national biomedical research priorities and preventing potential harm to relatives, Equally unavailable would be data vital to the determination of paternity and the identification of criminals in court cases. In addition, it would be impossible to conduct research on genetic diseases. The benefits, however, must be weighed against the fact that unrestricted access to genetic patient data would violate the autonomy of individuals to reveal only the personal information of their choice. Two models illustrate the ways in which health records are treated: the physician-patient model and the public health model.

THE PHYSICIAN-PATIENT MODEL

The precedent for confidentiality in the physician-patient relationship was set many years before the Hippocratic oath was written (Walters, 1983), and since that time, physicians have held to an ethical code of privacy in matters relating to patient's records.

Utilitarian Justifications.--One way to consider the privacy of the physician-patient relationship is utilitarian: for the physician to effectively treat the individual there must be trust between them. A patient can only be expected to reveal delicate health issues to the physician if the information is to be held in strict confidence. In daily life, a person can control whether or not to disclose personal information to others. One's private thoughts may be represented by a set of concentric circles, with the outermost circles containing information that a person is willing to give to anyone, such as height or occupation, and the innermost circles containing personal information that is reserved only for those closest to him or her, if anyone. In the medical model, a patient allows a physician to enter an inner circle in order to get help with a medical problem, and the physician therefore owes a duty to the patient to keep the information confidential (Walters, 1983). Certain types of genetic patient data may be considered so proprietary that, "Doctors in whose records this information may reside should hold it extremely confidential and should not keep it in the person's general medical file" (Wexler, 1983).

Patient Rights.--Another approach to the physician-patient relationship is centered on the rights of the individual. These rights become particularly important in considering the difference between collecting a family history and performing genetic tests. A patient has direct control over whether to provide a family history to a physician, while genetic testing can be performed on blood, body fluids, or tissues. This technical ability to collect genetic patient data raises two main concerns. First, the patient does not exercise the same discretionary control over information garnered from biochemical testing as he or she does in relating a family history: the patient merely assents or dissents to undergoing the test. Second, blood or tissue samples collected at other times for other rea-

sons may be tested genetically, without the knowledge of the patient.

Even following the guidelines for informed consent, with the patient agreeing to genetic tests, their technical nature increases the risk that the patient does not fully understand the possible significance of the data. Patients may also fail to anticipate the potential harm disclosure might cause him or her. The consent of the patient is required to remove blood or tissue from his or her body, and also to perform tests, but it is important that the patient be informed of all the tests which are done and that a concern for the privacy of the patient extends to the control of tissues removed from his or her body.

Under normal circumstances, health records are not released to third parties, except with the consent of the patient, so that medical information which exists in the record is still under the control of the patient. Nevertheless, current practices involving information release allow little or no control over withholding parts of data. A patient with a genetic trait or disease is rarely able to release only the parts of his or her record that do not contain that information once a waiver is signed, as those waivers are considered as 'blanket' consent for release of their entire medical record. However, even in instances when the physician-patient relationship can be maintained, there are several cases which supersede it and these can be grouped and called the public health model

THE PUBLIC HEALTH MODEL

A physician's duty to protect the privacy of his patient may be superseded by his duty to prevent harm to others, such as the patient family or society in general. For example, a physician must report the occurrence of cases involving gunshot wounds, battered children, and certain communicable diseases (Green and Capron, 1974; Walters, 1983). Government interest in reporting communicable diseases centers on identifying both the disease and those individuals who are at risk of contracting it, and mobilizing efforts to prevent or treat it. With certain communicable diseases, such as gonorrhea, there is a high risk of danger to significant numbers of people, and government involvement may be a way to reduce the risk. With gunshot wounds, it is possible that the injury occurred as a result of an illegal act that may place others at danger, and so government action may prevent harm to others. This concern for the public well-being often places the physician in a difficult ethical position, having to choose between the privacy interests of his patient and the interests of society. This is especially true in the case of psychiatrists who may have reason to believe a patient may become violent, and they must decide whether their belief justifies reporting the patient to the police (Walters, 1983).

Since there are many different issues involved in the disclosure of information, it is instructive to look at several different cases of the disclosure of information, beginning with the disclosure to the patient, himself.

DISCLOSURE TO THE PATIENT

The doctrine of informed consent, so called, was initially developed to assure a patient's self-determination and right to decide whether to undergo health care procedures. One of the most important arguments for an informed patient is that only with adequate information can an individual make informed decisions concerning his or her health or lifestyle, and genetic information can play an important role in these decisions. Another, recently discovered, and perhaps more compelling argument is that informed consent may actually provide numerous physical and psychological benefits to the patient (Andrews, 1984a).

Studies of elective surgery patients have provided the most notable evidence of the beneficial effects of information disclosure. Patients 'briefed' on the nature of surgical procedures and postoperative sensations exhibited a greater capacity to adjust to postoperative stress, needed less pain medication, and had fewer recovery days in the hospital. In another study of hospital patients, one of the chief reasons for refusing treatment seemed to be the occurrence of unexpected procedures which exacerbated patient uncertainty and aroused patient anger (Appelbaum, 1982).

However, the therapeutic effects of information disclosure are not limited to surgery patients. Patients scheduled for endoscopic examination— where a fiberoptic tube for internal viewing is placed down the esophagus and into the stomach—heard a taped description of the sensations frequently experienced during the procedure and subsequently needed less medication to tolerate the examination than those who did not hear the tape. Similar results indicating the benefits of disclosure have been found in studies involving blood donors, burn treatment, and sigmoidoscopy examinations (Andrews, 1984a).

Disclosure also acts as an informal check and balance system whereby a patient may reject a procedure that is being advocated more for the benefit of the practitioner than the patient. Although generally acting in the patient's best interest when they propose diagnostic procedures and therapies, physicians may be motivated by strong financial and professional considerations that place them in a conflict of interest (Schneyer, 1976).

Another potential benefit of informed consent is that it may enhance the quality of physicians' decisions. By

requiring physicians to provide clear and factual information about the risks and alternatives to a given procedure or therapy, they may recognize and account for their own judgment biases and suggest a more thoroughly considered course of action. Additionally, in the course of the physician describing a procedure, the patient may reveal information pertinent to the treatment choice— information which may result in a different choice of action.

There is no consistent or prescribed amount of information due the patient on a national basis, but there are three measures by which the legal system generally determines the patient's right to decide. One is the Reasonable Physician Standard, whereby the physician follows the standards of the community to determine how much, or whether to disclose anything to the patient. The second is the Reasonable Patient Standard, whereby the patient is informed of any and all information necessary or helpful to a reasonable patient. The third is the Individual Patient Standard, whereby the physician must take into account what he/she knows about the individual patient to determine what should be disclosed. Each of these standards carries different weight with different courts, and despite the widespread acceptance of the doctrine and its continued expansion, the patient's right to informed consent has always been and continues to be a qualified one (Andrews, 1984a).

Courts almost unanimously note several exceptions to the general rule: an emergency situation where the patient is unconscious or otherwise unable to authorize treatment, and serious damage will occur if treatment is not undertaken; where the patient is deemed incompetent to make a decision; where a waiver to informed consent is signed by the patient; and where therapeutic privilege is invoked because disclosure poses such a threat of psychological damage as to be unwise from a medical viewpoint (Andrews, 1984a).

Third party access

Several groups besides the individual would have an interest in genetic information gathered about an individual. For example, family members may wish to be alerted to potential health risks revealed by the genetic data about a close relative Also, insurance companies, employers, and the Federal Government have an interest in access to genetic patient data for various reasons which will be described below. In each case, there is conflict between third party access to information and the individual's right to privacy.

A physician's duty to protect the confidentiality of the patient data can be upheld if certain guidelines are followed when disclosing information to third parties:

- there should be a high probability of harm to others,
- the potential for harm should be deemed serious, such as being irreversible or fatal, and
- there should be reason to believe that the information will prevent harm. (President's Commission, 1983, p. 44).

Reasonable attempts for voluntary consent should be made, since it would not be ethical and may not be legal[4] to disclose information without the consent of the patient, and only the relevant information should be disclosed. These guidelines will be considered in the following situations: disclosure to family members, insurance companies, employers, and the government.

DISCLOSURE TO FAMILY MEMBERS

There are many situations in which genetic data about an individual may affect decisions made by close relatives. Genetic data may be of greatest importance to one's spouse or prospective spouse because it may directly affect the couple's reproductive decisions. The reason for disclosure is to prevent direct harm to the unborn and indirect harm to one's spouse. In many cases, one partner would wish to inform the other about possible genetic risks so that together they may make an informed decision about having children. In other situations, the affected partner may prefer not to inform the other, in order to avoid being identified as the cause of having deformed children or being the reason for not having children at all.

Disclosure to a spouse may indeed prevent harm if the couple decides not to have children at high risk of genetic disease. The reasons supporting disclosure of genetic patient data to a spouse increase with both the severity of a potential genetic disease and the probability of the children inheriting it.

Another reason for disclosure goes beyond reproductive decisions to include the need for the spouse and family to know the genetic condition of the affected person in order to make plans to care for them, both physically and financially. For example, if it were known that the provider of a household would develop polycystic kidney disease or Huntington disease, the family would have to plan for the debilitating effects of the disease, significant medical expenses, and future loss of income.

[4] A physician who discloses medical data to relatives or third parties may be sued for damages resulting from violation of the patient's privacy

Since children receive half their genes from each parent, they also have an interest in the genetic data of their parents. The case for disclosure to children is strong because there may be a significant probability of harm that could be reduced if the children were to take health precautions. In families with colonic polyposis, for example, those with the disease are at high risk of developing colon cancer, and preventive removal of the colon can thwart almost certain death from cancer. Knowledge about colonic polyposis can, therefore, be of extreme importance to those at risk.

Genetic patient data may also be relevant to health care of other relatives. In families that carry the gene for retinoblastoma, for example, children are at high risk of developing potentially fatal eye cancer. Knowledge that a relative has the disease may precipitate more careful scrutiny of cousins and siblings who are also at risk, thus potentially saving lives.

The case for access to more distant relatives is generally not as strong as for the immediate family, since the predictive value is lower, but here, too, genetic patient data might alert the person to potential health risks. If the severity of the disease and the degree of risk is high and action can be taken to prevent harm, then disclosure to more distant relatives maybe justified,

Finally, genetic patient data can be of use to children and other relatives of parents affected with a genetic disease when considering reproductive decisions. Prospective parents may choose not to bear children or may take special steps to monitor their children as a consequence of information obtained about diseases that are more likely in their children than in the general population.

DISCLOSURE TO INSURANCE COMPANIES

The insurance industry is the second largest user of medical information in the United States, after the Federal Government (Baskin, 1978). Both life and health insurance companies use medical information in order to assess the probability of health events for those who are insured. There is a great deal of variation between individual firms in the amount of information required to accept an applicant.

Health Insurance Companies.—One hundred ninety million people in this country had some form of health insurance coverage in 1983 (Health Insurance Association of America, 1984), and many people consider health insurance to be a necessity. The majority of health insurance policies are group policies, received in conjunction with employment. These group health policies do not consider the health risks of the applicants to determine their insurability or their premiums. However, claims made on preexisting health conditions are exempted for a period usually **of 30** to **120 days** (Health Insurance Association of America, 1984). The access of insurance companies to genetic patient data, therefore, does not seem to be an **issue for most group health insurance coverage .**

Individual health insurance policies, however, are similar to life insurance policies, since they both use medical information to determine the premiums. Group health insurance policies, generally used in employee benefit packages, usually require applicants to sign a blanket waiver permitting access to their entire health record, including family history and any genetic patient data.

The people who purchase individual policies include those over the age of 65, the self-employed, and workers in small businesses. The unemployed do not qualify for group insurance and usually cannot afford individual policies. For the remainder of this paper, the term "insurance" will encompass both life and individual health insurance.

Life Insurance Companies.—Most life insurance companies require an applicant to answer several questions about his or her health on an application form, and then if the answers warrant, and if the coverage sought exceeds a certain amount, they may require the applicant to release his or her medical records, submit to a medical examination, or both. The results of these medical findings and other data are then used to determine the life insurance premiums for an individual, or whether the person is insurable at all. Some of the questions are related to conditions with a genetic component, such as sickle cell disease, and if an applicant reports or displays the symptoms it is unlikely that he or she will be insured. Likewise, an applicant may be asked about the presence of heart disease, high blood pressure, or stroke in his or her immediate family, and an affirmative answer would increase the risk factors involved, even though the genetic basis of these diseases is not clear. The use of this genetic patient data raises several ethical questions that are not new, but the potential increase in the amount of genetic patient data in the future may increase the significance of these issues.

Risk Classification. -Insurance companies generally use several factors to determine an individual's insurance premium, such as gender, occupation, weight, and blood pressure (Cummins, et al., 1983). Recently, some insurance companies have begun using lifestyle factors, such as one's smoking or exercise habits, in assessing insurance risk.

***Controllable Risk* Factors.—Smoking is considered largely a voluntary activity, controllable by the individual, with strong actuarial evidence of significantly reduced life spans. It is also generally accepted that the primary health effects from smoking (e.g., cardiovascular disease, emphysema, and lung, esophageal,**

and bladder cancers) can be caused by smoking. Perhaps the major drawback of using this type of lifestyle information is that it is self-reported and, therefore, not verifiable; since there is a price incentive to report that one is a non-smoker, an applicant may not be truthful.

Diet is also a known risk factor for development of certain types of diabetes, arthritis, and susceptibility to colonic and breast cancers. Alcohol ingestion is associated with liver cirrhosis, esophageal and stomach cancers, and more than a dozen neurological syndromes.

Uncontrollable Risk Factors.—Until a 1983 Supreme Court Decision, it was common practice in the insurance industry to use the gender of the applicant to determine the premium. While that practice continues in the underwriting of individual policies, it is no longer allowable in group health, or employee benefits, policies. (I. Katz Pinsler, ACLU, personal communication, 1984). In contrast to lifestyle factors, one's gender is genetically determined and is not under one's voluntary control, but there is strong actuarial evidence that women tend to live longer than men. Gender is verifiable, which makes it relatively easy to use as a determinant. Some actuaries, however, question the use of gender, claiming that other factors such as smoking, lifestyle, work habits, or competitive behavior maybe the cause of the mortality differences (Cummins, et al., 1983 p. 86), *(Business Insurance,* 1981).

The race of an applicant is not used to determine the premium, although the criteria are similar to the case of gender: one's race is not under one's own control, but although there is actuarial evidence for mortality differences between races, it is difficult in practice to identify distinct races because of the degree of racial mixing. Insurance companies argue that the actuarial differences between races are due to socioeconomic differences and not to race, per se, and that these factors are already considered in the actuarial process. Also, several States have prohibited the use of race in insurance underwriting (Cummins, et al., 1983 p. 90).

Some factors with genetic components are used to determine the insurance premium, such as a family history of heart disease. The criteria for using genetic patient data are similar to those for race and market since one's genetic complement is not voluntary. At present, however, most genetic diseases cannot be verified before they are expressed.

Efficiency and Equality.—Insurance companies, as profit-maximizing firms, have an incentive to use any readily available genetic patient data because it will allow them to function more efficiently in the free market. By using this information, they will be better able to identify high-risk applicants and thus be able to charge them proportionately higher premiums.

The adverse selection model, described below, provides one explanation for why insurance companies might wish to use genetic patient data in the underwriting process. In an insurance market, when there is no distinction made between the risks of the applicants, there is a tendency for those who know they are at risk to purchase the highest coverage they can afford. With more of these high-risk clients, insurance company costs will increase, because the company will be paying more claims. The increase in costs tend to drive up the insurance premiums, causing low-risk clients to leave, this results in a pool of high-risk clients, paying high premiums. If another type of insurance were available that differentiated applicants on the basis of risk, insurance companies could make a profit by offering it as an option (McGill, 1984). The use of genetic patient data could help insurance companies counter this adverse selection phenomenon which can lead to high rates.

The question of fairness remains, however, and the crux of the issue is whether it is more fair for those individuals with high risks to pay proportionately higher rates or for all individuals to pay the same rate, regardless of risk. In the first case, market forces will act to differentiate people on the basis of the risk they present to the insurance company, and may lead to groups of individuals unable to purchase insurance at an affordable price. This type of situation may seem fair when it concerns something over which an individual has some control, such as one's smoking habits, but the fairness issue becomes more difficult when it involves something over which one has no control, such as one's genetic complement.

In the latter case, where everyone pays the same rate, low-risk individuals would be subsidizing high-risk ones. In either case, one group will be harmed, and society needs to determine whether the low-risk or high- risk individuals will bear the burden. A compromise could be made using the U.S. Social Security system as a model. In this system, contributions are not actuarially equal to benefits, but the level of benefits is related to the amount contributed (Cummins, et al., 1983).

Impacts of Using Improved Genetic Patient Data.—Increased screening for genetic diseases could lead to numerous groups of individuals that are substandard risks, uninsurable, or who must pay prohibitively high rates. At present, diseases or health conditions that already exist carry more weight in the underwriting formulae than those conditions which are just statistical probabilities. If reliable genetic patient data were

available at low cost to use in insurance underwriting, however, more weight might be placed on them. For example, if an applicant has expressed polycystic kidney disease, he or she is likely to be denied insurance. However, since this disease is not expressed until later in life, an individual can carry the gene for the disease and still obtain insurance, since there is no way to detect the gene at present. If the gene could be detected at an early age and one could say with high certainty that a person would develop polycystic kidney disease, then such tests might be used to determine insurability. Further questions arise concerning the use of tests that are under development, are not perfectly accurate, or are prohibitively expensive. For example, if a test were developed which indicated the presence of a gene but not whether it would result in disease,[5] should the results of the test be used in the underwriting process? Three States, Florida, Maryland, North Carolina, (Case, Health Insurance Association of America, personal communication, 1984) already specifically prohibit health insurance companies from discriminating against sickle cell carriers.

The increased use of genetic patient data in the underwriting process has significant legal implications. Since several genetic diseases are linked closely with race, (see table B-1) if an insurance company uses genetic patient data to compute the health risks of applicants, it would have a disparate impact on the affected races. As genetic markers become more refined, it may become increasingly difficult to separate the prevalence of specific genetic diseases from race. Therein lies a potential conflict with current or future civil rights laws.

The role of Federal and State Governments in constraining access to genetic patient data may increase in proportion to the amount readily available. Patient protection will be afforded by case law, but some aspects of how genetic patient data are specifically handled (in contrast to other personal or medical information) may depend on new Federal or State regulations. Public policy on genetic patient data turns, in part, on whether it is classed as a basis, like race, for civil rights protections. As the availability of genetic patient data grows, pressures to use it and disclose it to third parties will also likely increase. Legislatures may wish to consider new laws to redress misapplications or to cover areas not clearly defined in case law.

DISCLOSURES TO EMPLOYERS

Because of the significant costs of occupational illness— including the time lost from work, the cost of training replacements, and increased health insurance rates—a profit-maximizing company has an incentive to reduce the incidence of work-related disease as long as the costs of the reduction are lower than the costs of the disease (Murray, 1983).

Because the expression of a genetic disease is frequently thought to be determined by a combination of genetic and environmental factors (Harsanyi, 1981), companies may have the ability to change specific environmental factors which otherwise enhance the possibility of disease expression. Availability of genetic data on employees could then lead to companies assisting those employees in remaining healthy.

But what if the cost of the disease is higher than that of the reduction? Or if there is no known way to reduce incidence? Or if a company is disinclined to institute changes due to either inconvenience or cost? The use of genetic patient data under these circumstances could lead companies to discriminatory hiring, promotion, or lay-off policies. In this light, the question once again arises as to whether companies should have general access to genetic patient data.

Title VII of the amended 1964 Civil Rights Act, and sections 503 and 504 of the 1973 Rehabilitation Act govern employment rights. The former prohibits employment discrimination on the basis of race, color, religion, sex, or national origin. The latter prohibits discrimination against otherwise qualified handicapped individuals by employers who are Government contractors or recipients of Federal assistance.

Currently, the term 'handicapped individual' is defined in section 503 as "any person who: 1) has a physical or mental impairment which substantially limits one or more of such person's major life activities, 2) has a record of such an impairment, or 3) is regarded as having such an impairment." Equally, in section 504, an employer receiving Federal financial assistance may not make preemployment inquiry about whether the applicant is handicapped or about the nature and severity of an existing handicap unless a preemployment medical examination is required of all applicants and the information obtained from the examination is relevant to the applicant's ability to perform job-related functions. Both sections serve to limit the use of discriminatory preemployment examinations and tests, but it must nevertheless be determined whether genetic trait is a handicap and whether screening procedures are job related.

These statutes indicate that individuals are not to be discriminated against on the basis of some immutable characteristics and that their abilities are to be judged on an individual basis. Since genetic screening could result in employment discrimination against groups of individuals with particular inherited traits, one question that arises is whether such discrimina-

[5] Since most diseases are due to a combination of genetic and environmental factors, genetic tests may eventually prove to be mostly of this type

Table B-1.—Genetic Diseases Found in Higher Prevalence Among Specific Racial or Ethnic Groups

Condition	Prevalence
Amyloid nephropathy associated with familial Mediterranean fever	1:3,000Sephardic Jews
Aspartylglycosaminuria	70-100 cases in Finland
Cystic fibrosis	1:2,000 Caucasians
Diabetes mellitus, type 2 (insulin-dependent, ketosis-resistant)	1:130 Caucasians, uncertain in blacks
Dubin-Johnson syndrome	1:1,300 Iranian Jews
Essential fructosuria	1:130,000; more common in Jews
Galactosylceramide lipidosis (globoid cell leukodystrophy; Krabbe's disease)	1:50,000 in Sweden
Gaucher's disease, type I	1:2,000 U.S. Jews
Glucose-6-phosphate dehydrogenase (G6PD) deficiency; multiple allelic disorders, including mild A-type and severe Mediterranean type	A-type: 1:11 U.S. blacks (males) Mediterranean type: common in Africa, Middle East and other Mediterranean countries
Gyrate atropy of the choroid and retina	1:50,000 in Finland
Hereditary fructose intolerance	1:20,000 in Switzerland
Hereditary spherocytosis, several types	1:5,000 Caucasians
Hermansky-Pudlak syndrome	1:60,000 Caucasians 1:5,000 Puerto Ricans
Intestinal lactase deficiency	1:10 Caucasians, majority of Asians, Africans, and U.S. blacks are affected
Niemann-Pick disease	1:25,000 U.S. Jews
Nonketotic hyperglycinemia	1:250,000 in United States 1:12,000 in Northern Finland
Occulocutaneous albinism, tyrosinase-negative type	1:39,000 Caucasians 1:28,000 blacks
Occulocutaneous albinism, tyrosinase-positive type	1:37,000 Caucasians 1:15,000 blacks 1:150 in certain American Indians
Pentosuria	1:2,500 Eastern European Jews
Primary gout: idiopathic	1:500 in Western populations 1:50 in American males by age 50 1:10 in males in some Polynesian groups 1:25 in females in some Polynesian groups
Sickle cell anemia	1:500 U.S. blacks (newborns)
Tay-Sachs disease	1:3,000 U.S. Jews
Thalassemia, multiple allelic disorders	High frequency in Mediterranean, African, and Asian populations
Tyrosinemia, type I (hepatorenal tyrosinemia; tyrosinosis)	1:10,000 French Canadian isolate
Variegate porphyria	Common in South Africa; rare in other parts of the world
Xeroderma pigmentosum, multiple types involving multiple gene loci	1:25,000 in Egypt

SOURCE: Stanbury, 1983; as amended by Bowman, personal communication, 1984.

tion is prohibited by these two acts (OTA, 1983). If they are judged not to prohibit genetically based discrimination, another question raised is whether additional federal legislation will be forthcoming.

The 1970 Occupational Safety and Health Act (OSHA), which requires employers to maintain a workplace free from recognized hazards, does not specify the means by which that requirement can be met. For example, it neither supports the argument that genetic testing is required nor that genetic testing is prohibited. Although the results of genetic testing could have an adverse affect on particular employees, it certainly cannot be classified as a "hazard" (OTA, 1983). Yet genetic testing might become the basis for employment discrimination, or harm to employees.

In this light, it is significant to note that it is common practice for employees to sign a blanket waiver allowing the company to gain access to all medical records it deems necessary. Employees generally "have little genuine expectation of true confidentiality as to employment medical records" (OTA, 1983). Any duty to the confidentiality of the patient is based on a physician-patient relationship, and the traditional view is that a physician-patient relationship does not exist be-

tween an employee and an employer-provided physician. Some courts take a view that the existence of a physician-patient relationship is dependent on the context of the health care provided. If the physician-patient relationship does not exist, neither does the duty of confidentiality, and so the company generally may have access to the medical records of its employees.

There are also few common law restrictions on the disclosure of genetic patient data to parties outside of the company, except for several State and Federal restrictions. For example, California requires employers to establish procedures to protect the privacy of medical records, and records may not be released without the consent of the employee. Because of the potential harm to the employee arising from disclosure, legislators may wish to anticipate the outcome of the increased use of genetic information by employers.

Unauthorized Access.—Because of the use of computers to maintain health records, there has been a growing concern for the security of the information, especially in light of the reports of computer crime. These concerns are not unique to the health care field, since every major sector of the economy is relying more on the computer for the maintenance of records. Genetic patient data may not be as obvious a candidate for computer theft as would be valuable trade secrets, but patient records at Memorial Sloan-Kettering Cancer Center have already been broken into (Marbach, 1983), and so the possibility of unauthorized or inadvertent access should not be discounted as greater amounts of genetic patient data become stored.

One solution to the problem of unauthorized access is to remove any identifying data from the record and keep it in a separate file. Then codes could be used to match the individuals to their records. Another solution is to extend the concentric circle model of privacy to include the genetic patient data stored in computer files. The information could carry different access codes, so that information could be accessed only by those physicians who need to know. Different individuals would therefore have access to different levels of private information, but this would not obviate the need for patient control of disclosure of information to third parties (Walters, 1983). These safeguards, while protecting the privacy of individuals, might also have the detrimental effect of making it more difficult for physicians to use the information in the medical record. The experience of research on Huntington Disease suggests that it is possible, by careful attention to data entry and access restriction, to provide aggregate data while protecting individual privacy (Wexler, personal communication, 1984).

DISCLOSURE TO THE GOVERNMENT

The government will likely play an important role in issues relating to genetic patient data both as a significant user of information and as a body acting to control the access to that information.

The major objective of government in using medical information is the protection of the public health. For example, by collecting statistics on the frequency and incidence of various diseases, the government perhaps can take measures against those diseases in the future, perhaps by mobilizing health care efforts in particular areas. Other likely government uses of genetic patient data include:

- providing information about medical costs,
- developing policies to better allocate health resources, and
- identifying diseases which merit additional research.

For these purposes, the identity of the individual is not important, and so all identifying pieces of information can be culled from the record. For other purposes, such as tracking individuals with specific genetic diseases or doing epidemiological research, however, it is important to know the identity of those at risk. In the interests of privacy and security, the records may be coded and the identifying information may be stored in a separate file, but the identity of individuals must still be accessible. In this instance, privacy can be retained by authorizing only one, or a few, disease centers to follow individual patients.

Because of the growing amount of information collected and used by the Federal Government, and because of improvements in information storage and retrieval technologies in the foreseeable future, Congress passed the Privacy Act of 1974 to set a policy for the appropriate use of personal information. The Act states that "The right to privacy is a personal and fundamental right protected by the Constitution of the United States, " and that in order to "protect the privacy of individuals identified in information systems maintained by Federal agencies, it is necessary and proper for the Congress to regulate the collection, maintenance, use, and dissemination of information by such agencies" (Privacy Protection Study Committee, 1977). The Act describes in detail the conditions of disclosure and access, as well as agency requirements and rules. The Act forbids the disclosure of any records to any person or agency, except with a written request by, or with the prior request of, the individual to whom the record pertains. Violations of the Act can lead to a civil liability.

The Federal Government is involved in providing funding for genetic testing and counseling, thus assisting in the process of collecting genetic patient data. The government can also serve as an effective forum to discuss the ethical, legal, economic, and social aspects of genetic information. Since there are many different groups involved in balancing the issues of privacy and access, the Federal Government can ensure that these issues are included in the decisionmaking process.

The States also have the authority to compile and store genetic patient data that is of potential benefit to the public health (Reilly, 1977 pp. 250-252). Several States have written laws that regulate the type of information that can be collected and the procedures through which disclosure can be made (Reilly, 1977 pp. .252 -256) The States also have control over the business practices of various industries including insurance companies, and they may determine the propriety of using genetic patient data in different employment and underwriting situations. Some States have already forbidden the use of such data as gender, age, handicaps, or other impairments in the underwriting process (Cummins, et al., 1973).

General education is an issue of Federal, State and local interest, necessary so that all people can have an understanding of genetics sufficient to understand the complex issues of genetic patient data (President's Commission, 1983; Rowley, 1984). Some schools have responded to this need by making genetics a major focus of their biology courses. Genetic education is an issue for health care providers, as well. The teaching of genetics occurs primarily' during the first 2 years of medical school, with little integration of genetics into the practical side of clinical training (Rowley, 1984). As the technology for identifying genetic disease improves, it is important that physicians become aware of that technology and how to use it with patients.

Conclusion

Public policy on genetic patient data is centered on determining the rights of privacy and access, pitting individual autonomy against relatives' or third parties' needs for information. The legitimacy of others' needs are determined by the potential benefits to relatives, health providers, insurers, employers, or the general public compared to potential harm to the patient from disclosure, Several factors are included in such assessments, including the seriousness of the genetic condition, the genetic relationship between interested parties, and the probability of preventing harm or promoting good by disclosure. When no genetic relationship exists, as in the case of insurers and employers, issues of fairness arise. Continuing public scrutiny may be instrumental in the evolution of deciding on a hierarchy of conditions and people for whom disclosure of genetic patient data is important (Rosenfeld, 1984).

Public policy on genetic patient data attempts to control access so that individual privacy is protected. This effort may include support of data storage methods that are coded so that epidemiologic research and research priority assessment may be performed without jeopardizing individual privacy. Legislation may be required to guide genetic data collection agencies in what constitutes appropriate disclosure of information and to act as a deterrent to unauthorized access. Public policies may be required that would strengthen individual's control over access to their genetic data. In contemplating new legislation, care must be taken to ensure that controls are not so strict that the genetic patient data cannot be used for legitimate and lifesaving purposes.

Working Group on Human Gene Therapy Recombinant DNA Advisory Committee National Institutes of Health

LeRoy Walters, *Chairman*
Director, Center for Bioethics
Kennedy Institute of Ethics, Georgetown University

W. French Anderson
Laboratory of Molecular Hematology
National Heart, Lung, and Blood Institute
National Institutes of Health

Judith Areen
Georgetown University Law Center

Alexander Capron
The Law Center
University of Southern California

James F. Childress
The Wilson Center
Smithsonian Institution

Samuel Gorovitz
Department of Philosophy
University of Maryland

Susan K. Gottesman
Laboratory of Molecular Biology
National Cancer Institute

Clifford Grobstein
Department of Science, Technology, and Public Affairs
University of California, San Diego

Maurice J. Mahoney
Department of Human Genetics
Yale University

Robert E. Mitchell (ex *officio)*
Attorney at Law
Norwalk, CA

Arno G. Motulsky
Department of Medicine
University of Maryland

Robert F. Murray
Division of Medical Genetics
Howard University

Robert F. Rich
School of Urban and Public Affairs
Carnegie-Mellon University

Harold Varmus
Department of Microbiology
University of California

Anne R. Witherby
Public Representative

Executive Secretary

William J. Gartland, Jr.
Office of Recombinant DNA Activities
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Consultant

Howard M. Temin
Department of Oncology
University of Wisconsin

Liaison Representatives

Charles McCarthy
Office of Protection From Research Risks
Office of the Director
National Institutes of Health

Henry I. Miller
National Center for Drugs and Biologics
Food and Drug Administration

Appendix D

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Appendix E

List of Abbreviations and Glossary

Abbreviations

ADA — Adenosine deaminase, an enzyme whose absence leads to metabolic errors that in turn inhibit the bodies' immune defenses. ADA deficiency is a rare disorder caused by genetic mutation that is inherited as an autosomal recessive trait. It is not the same disorder as PNP deficiency, although there are some similarities.

cDNA — Complementary DNA, DNA made from a messenger RNA template (see Technical Notes).

DNA — Deoxyribonucleic Acid (see Technical Notes).

EAB — Ethics Advisory Board, established under the Secretary of Health and Human Services to advise the Secretary on ethical issues related to public policy. There can be one or more such boards (Code of Federal Regulations, 1983). None presently exist, despite Federal regulations.

HPRT – Hypoxanthine-guanine phosphoribosyl transferase (or hypoxanthine phosphoribosyl transferase), an enzyme whose complete deficiency leads to Lesch-Nyhan syndrome, and whose partial absence leads to gout. HPRT deficiencies are inherited as X-linked traits.

IBC — Institutional Biosafety Committee, established at a university hospital, private firm, or other research center. IBCs supervise research protocols to ensure compliance with Federal Guidelines for Research Involving Recombinant DNA Molecules. In the case of Human Gene Therapy, this will involve review also by the RAC and the NIH Director before approval to commence experiments.

IRB — Institutional Review Board, established at a university, hospital, private firm, or other research center. IRB's must be composed of 5 members, at least one of whose primary interests are in nonscientific areas and one member neither affiliated with the institution nor in the immediate family of anyone who is so affiliated. IRBs supervise research protocols to ensure compliance with Federal Human Subjects Protections, and report noncompliance with the Protections to appropriate institutional officials and the Secretary (Code of Federal Regulations, 1983).

mRNA — Messenger RNA (see Technical Notes).

NIH – National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services.

OCT – Ornithine carbamoyl transferase (or ornithine transcarbamylase), an enzyme that mediates metabolism in the urea cycle, and whose deficiency is inherited as an X-linked trait.

OSTP – Office of Science and Technology Policy, reporting directly to the President.

OTA – Office of Technology Assessment, U.S. Congress.

PKU – Phenylketonuria, a disorder caused by deficiency of an enzyme, phenylalanine hydroxylase, that metabolizes one amino acid (phenylalanine) to another (tyrosine). It is inherited as an autosomal recessive trait.

PNP – Purine Nucleoside Phosphorylase, an enzyme whose absence leads to metabolic errors that in turn inhibit the bodies' immune defenses. PNP deficiency is caused by a rare genetic mutation inherited as an autosomal recessive trait different from ADA deficiency.

RAC – Recombinant DNA Advisory Committee, constituted at the National Institutes of Health to advise the Director of NIH on experiments involving recombinant DNA and molecules derived from recombinant DNA.

RFLP – Restriction fragment length polymorphism, a phenomenon involving variation in the length of DNA cut by specific enzymes that permits location of genes of interest, including disease-related genes (see app. A).

RNA – Ribonucleic Acid (see Technical Notes).

tRNA — Transfer RNA (see Technical Notes).

TSD — Tay-Sachs disease, an autosomal recessive disorder caused by deficiency of the enzyme hexosaminidase A.

UCLA — The University of California at Los Angeles.

Glossary

Achondroplasia—a defect in the formation of cartilage at the ends of long bones (femur, humerus) that often produces a type of dwarfism. There are a number of hereditary forms, the most common of which is an autosomal dominant.

ADA deficiency—an autosomal dominant disorder caused by deficiency of the enzyme adenosine deaminase, and resulting in inhibition of the bodies' defenses.

Allele-one of several possible alternate forms of a given gene.

Alpha fetoprotein—a fetal protein found in amniotic fluid that indicates, by its presence and concentration, the presence of certain fetal defects (e.g. anencephaly; spina bifida).

Alpha globin mRNA deficiency—an insufficiency in the messenger RNA coding for the alpha chain of hemoglobin.

Alpha-1-antitrypsin deficiency—a recessive heritable disease due to the lack of a protein inhibiting enzyme, alpha-1-antitrypsin. Death is usually due to degenerative lung and liver disease.

Alpha thalassemia—an hereditary disease due to an insufficiency in the number of alpha hemoglobin molecules in the blood. It is usually caused by the deletion of a portion of the gene coding for the alpha hemoglobin molecule.

Alzheimer disease—a progressive brain disease marked by progressive dementia (loss of memory and higher mental functions) and associated with characteristic changes in and near nerve cells: senile plaques and neurofibrillary tangles. The evidence suggests the disease can be caused in several different ways: a hereditary form exists, but its prevalence is uncertain; slow acting infectious agents may play a role; the body's immune system may react against the brain; specific populations of nerve cells may die; and environmental toxins (ionic aluminum or silicon) may be involved; or some combination of factors.

Aminoaciduria (branched chain; and ketoaciduria)—any of a large class of diseases marked by the accumulation of various amino acids (branched chain or ketoacids) in the blood. Symptoms vary with the specific compounds involved, each presumably the result of different defective enzymes in the relevant metabolic pathways.

Amniocentesis—the process of withdrawing a sample of the amniotic fluid surrounding the fetus in utero through a needle into a syringe. The fluid taken (usually 2 to 8 milliliters, or cubic centimeters) contains cells shed by the developing embryo. These can be grown in cell culture and either analyzed biochemically or cytogenetically to detect a variety (over one hundred) of hereditary diseases.

Anencephaly-–a congenital defect characterized by the absence or extreme reduction in size of the brain and spinal cord. It is usually due to complex developmental malformations rather than a simple genetic defect.

Antibody molecule-protein molecules manufactured in the body that serve to recognize and destroy cells identified as foreign. The antibody molecule is a tetramer, composed of two large, heavy chain molecules and two light (kappa or lambda) chain molecules. The ability to bind to different antigens (molecules that stimulate the production of antibodies) resides in antibodies.

Antigen—a molecule, usually a large protein or carbohydrate, which when introduced into the body stimulates the production of an antibody that will react specifically with the antigen to remove it.

Aneuploidy—a defect of chromosome number. Normal sexual organisms are diploid; that is, they have two complete sets of chromosomes, one of paternal origin and one of maternal origin. Defects of ploidy can be either of individual chromosomes, where one more or one less is present than normal (trisomy; monosomy), or of entire chromosome sets (e.g., triploidy).

Argininemia—a recessive genetic defect marked by severe mental retardation and various neurological disorders. It is due to an excess of arginine in the blood and spinal fluid, this being caused by decreased activity of the enzyme (arginase) that normally degrades this molecule. It was suggested a decade ago that argininemia could be treated in humans by deliberate infection with the Shope rabbit papilloma virus, which had been shown to restore arginase activity of deficient cells in tissue culture.

Arginosuccinate synthetase deficiency—see Cit*rullinemia.*

Arteriosclerosis (hardening of the arteries)-–a condition in which the walls of blood vessels become thickened and hardened due to a number of different pathological conditions. The causes are multiple and complex, and often incompletely known. There is good evidence that genetic factors are sometimes involved.

Arylsulfatase B deficiency—an autosomal recessive disorder of lipid metabolism caused by a deficiency in the production of the enzyme arylsulfatase B. A form of metachromatic leukodystrophy, the symptoms are severe physical changes including hydrocephalus, with death usual by the late teens.

Atherosclerosis—the most common form of arterio-

sclerosis in which there are localized deposits of fatty material (lipids) in the walls or the chamber (lumen) of blood vessels. It can be the result of defects in lipid metabolism, many of which are genetic in nature.

Auto-immune disease—a disease in which the body's defenses fail to distinguish its own tissue from foreign matter ('(self" from "non-self") and attack it. The causes are probably errors in gene regulation, and there are clearly hereditary forms of this disease. A common form is lupus erythematosus, in which the connective tissues of the body (collagen especially) are progressively destroyed.

Autosomal dominant—a genetic trait (or a gene) carried on one of the autosomes that produces an observable phenotype even if present in only one copy (i.e., of the two alleles present for any given gene, if only one of them is a dominant it will be expressed regardless of whether the other is dominant or recessive).

Autosomal recessive—a genetic trait (or gene) carried on one of the autosomes that must be present in two copies (both of the alleles present must be of the same type) in order for the gene to be expressed and the trait seen in the phenotype.

Autosome—any chromosome other than the sex chromosome.

Azacytidine (5-azacytidine)-a drug used in cancer therapy that has also been used experimentally to promote expression of hemoglobin F genes (to replace defective Beta globin genes) in patients with thalassemia and sickle cell disease.

Bacteriophage (phage)—a virus that infects a bacterial cell. Phage consist of a core of genetic material (DNA or RNA) carrying the particle's genetic information which is surrounded by a protein coat or capsule. When a phage infects a host cell, the cell machinery that manufactures protein in response to genetically encoded instructions is commandeered by the phage and used to produce offspring phage. These are released when the bacterium dies, liberating from 100 to 10,000 new phage particles per infected bacterium.

Beta globin--one of the several types of hemoglobin molecules. In normal adult humans hemoglobin is a compound molecule formed of four protein subunits (globins) and a heme group. The four globins consist of two alpha and two beta molecules.

Beta thalassemia—a hereditary genetic defect caused by a deletion or alteration of a portion of the gene coding for the beta globin molecule. The result is an insufficiency in the number of beta globin molecules, which leads to abnormal hemoglobin,

Blastocyst—the developmental stage (in a mammalian embryo) immediately following the morula. It consists of an outer layer (the trophoblast) containing a cell mass attached to the inner wall of the interior cavity, or blastocoele. (See Technical Notes.)

Carcinogen—an agent or chemical that causes cancer.

Carrier (silent carrier)—an individual carrying a genetic defect and capable of transmitting it to offspring, but who does not show the defect him/herself. Most often, a carrier is heterozygous for a recessive allele, that is, carries only one of the two copies of a gene necessary for the trait to be manifest. It is possible, however, for an individual to carry a dominant allele that is not expressed and thus to transmit the trait to offspring while never showing it him/herself.

Chorionic villus biopsy—a technique of ante-natal diagnosis by which a sample of tissue is taken from the placenta (whose cells are of fetal origin) and analysed to detect the presence or absence of certain hereditary defects *in utero.*

Chromosomal disorders-any of a great variety of pathological conditions associated with abnormalities of the chromosomes, whether of number (aneuploidy) or structure (insertions, deletions, rearrangements).

Chromosome (colored body)--so named by early researchers because they stained very darkly when colored with certain dyes, chromosomes are the location of hereditary (genetic) material within the cell. This hereditary material is packaged in the form of a very long, double stranded molecule of DNA surrounded by and complexed with several different forms of protein. Genes are found arranged in a linear sequence along chromosomes, as is also a large amount of DNA of unknown function, but that may serve simply to help keep one gene separated from its neighbors.

Citrullinemia--an autosomal recessive defect whose clinical symptoms are associated with a deficiency in the enzyme argininosuccinate synthestase. Symptoms include ammonia intoxication, severe vomiting, and mental retardation.

Cleavage–the stage of cell multiplication immediately after fertilization of the egg. It lasts until the cells begin to segregate and differentiate, producing a blastula and then gastrula.

Complementary DNA-- (cDNA)–DNA synthesised from a messenger RNA template rather than the usual DNA template. cDNA is often used as a DNA probe to help locate a specific gene in an organism. The advantage of cDNA over mRNA as a probe is that the mRNA can be used to identify a specific gene product (e.g., an enzyme important to the

cause of a hereditary disease) and then to produce a DNA probe (more stable and more easily handled than RNA) to find the gene responsible for the hereditary disease.

Conceptus—a fertilized egg; an egg after conception.

Cystic fibrosis—an autosomal recessive disorder in which the glands do not function normally. Most often seen in children and young adults, it is usually lethal. Death is due to excess mucus in the lungs and pancreatic insufficiency.

Cytogenetics—the study of chromosomes and their behavior in the cell: what they look like, how many there are, how they are replicated and distributed to daughter cells (mitosis) or among gametes (meiosis).

Cytotoxic agents-chemicals, compounds or other agents that can cause cell death for any of a variety of reasons.

Dementia—loss of higher mental functions: memory, reasoning ability, speech, etc.

Diabetes mellitus—a disorder of carbohydrate metabolism marked by elevated blood sugar due to inadequate insulin production.

di-methyl adipimidate–an experimental compound used to prevent sickling in the red blood cells of patients with sickle cell anemia.

DNA (deoxyribonucleic acid)—the molecule containing hereditary information in all but the most primitive organisms (some viruses, that use RNA). The molecule is double stranded, with an external "backbone" formed by a chain of alternating phosphate and sugar (deoxyribose) units and an internal ladder-like structure formed by nucleotide base-pairs held together by hydrogen bonds. The nucleotide base pairs consist of the bases adenine (A), cytosine (C), guanine (G) and thymine (T) whose structures are such that A can hydrogen bond only with T, and C *only* with G. The sequence of each individual strand can be deduced by knowing that of its partner, This complementarily is the key to the information transmitting capabilities of DNA. (See Technical Notes.)

DNA probe–a molecule (usually a nucleic acid) of known structure and/or function that has been tagged with some tracer substance (a radioactive isotope or specific dye-absorbing compound) that is used to locate and identify a specific gene or region of a chromosome or portion of the genome.

Dominant--a gene that produces a visible effect even when present in heterozygous condition; each diploid cell contains two copies (alleles) of the gene at any specific locus. An allele that is expressed regardless of the nature of its companion allele is said to be dominant.

Down syndrome—a chromosomal disorder caused by the presence of all or part of an extra 21st chromosome. The symptoms are mental retardation, congenital heart defects, immune system abnormalities, various morphological abnormalities and a reduced life expectancy. Down syndrome is one of those diseases that has been most clearly shown to increase in frequency with advancing maternal age. (Down syndrome has been known by several equally inappropriate common names in different cultures, e.g. '(Mongolism" in the West and "round-eye" syndrome in the Orient.)

Drosophila—a genus of diptera, or two-winged insects, that has been extremely useful in genetic studies of nearly every sort. This is because of the unique collection of advantages afforded those working with the organism, which include a short generation time (so that many generations can be studied in a fairly short period of time) a high fecundity (thousands and even millions can be realistically studied in a reasonable length of time) and the extremely favorable giant polytene chromosomes in the salivary glands of the larvae, which make it possible to correlate genetic phenomena with morphological changes in the chromosomes, and follow these characters through numerous generations and experimental crosses. Also known as "fruit flies, " this genus is generally harmless, and not to be confused with the '(true fruit-flies" or tephritids, which are severe agricultural pests.

Duchenne Muscular Dystrophy—see *Muscular dsytrophy,* Duchenne type.

Dwarfism-a pathological condition of abnormally short stature. Some cases are known to be hereditary, while others result from disease or metabolic dysfunction.

Electrophoresis--a technique for separating different molecules based on their differential movement in an electric field. This differential movement is a complex function of molecule size, shape, and net electrical charge.

Embryogenesis—the process of cell growth that produces an embryo from the proper mixture of a zygote, nutrients, and time.

Expression—the process by which the blueprint contained in DNA is converted into the structures and biochemical mechanisms present and operating in a cell.

Expressivity—a term referring to the degree to which a gene is manifest in an individual. Genes for some traits (e.g., curliness of hair) may vary in the extent or severity to which they are seen in different individuals. Genes known to be manifest in different degrees in different individuals are said to show differential or variable expressivity.

Fabry disease—an X-linked (the gene is located on

the X chromosome) hereditary disease of lipid metabolism. Symptoms are a particular type of skin lesion, kidney disease (the usual cause of death) and a variety of neurological and biochemical abnormalities.

Fetoscopy--a procedure whereby the fetus is visually examined with a fiber optic instrument while still *in utero*.

Galactosemia--an inborn error of metabolism (genetic defect of an enzyme system) marked by the inability to digest galactose, a sugar produced (along with glucose) in the digestion of lactose, the common sugar in milk and dairy products. The symptoms of galactosemia are an accumulation of galactose and byproducts which leads to liver damage, cataracts, and mental retardation. Some relief can be achieved by limiting the dietary intake of milk and dairy products.

Gametes—mature male or female reproductive cells—sperm or ova. Gametes of the opposite sex, when fused, lead to the formation of a new, diploid organism.

Gamma globulin—a large protein molecule found in the blood that is very important to disease resistance. Individuals with a hereditary deficiency in the production of this molecule (gamma globulinemia) experience a decreased ability to withstand bacterial and viral infections.

Gaucher disease–an autosomal recessive defect of lipid metabolism found with higher frequency among Ashkenazic Jews of Eastern European origin and their descendants. Symptoms include enlarged spleen and liver and various neurological disorders. There are several different types, the two most common being a chronic adult form and an acute juvenile form that often leads to early death.

Gene—the portion of a DNA molecule that comprises the basic, functional hereditary unit; a sequence of DNA that produces a specific product. The fruit fly, *Drosophila melanogaster* probably has about 10,000 genes, whereas man may have as many as 100,000 genes.

Gene modification—a process of genetic therapy in which genes are altered in the living organism. It is not yet possible, but is expected in the future.

Gene supplementation—a technique of genetic therapy in which "new" or repaired genes are introduced into a cell by microinjection or a similar process.

Gene surgery—a procedure whereby a defective gene is excised and removed from a cell. A normal gene may be substituted.

Gene transplantation—a technique of moving an entire gene from one organism into another.

Genetic marker-any character that acts as a signpost or signal of the presence or location of a gene, chromosome, or hereditary characteristic in an individual, a population, chromosome or a DNA molecule. For example, the phenotype of male sex is a reliable indicator of the presence of the gene for H-Y antigen, a cell surface protein found in all genotypic males.

Genome—the total genetic information contained in an organism's genes. Also described as the total content of all the chromosomes in an organism.

Genotype—the total of the genetic information contained in the chromosomes of an organism. Compare to the phenotype, or external or morphological appearance of an organism. For example, an individual may have a heterozygous genotype for eye color consisting of an allele for brown eyes (which is dominant) and an allele for blue eyes (which is recessive) or a homozygous genotype, with two alleles (both dominant) for brown eyes. In either case, the phenotype is the same: brown eyes.

Germ line—also known as "germinal tissue, " it is the tissue or cell lineage that produces gametes and is used for reproductive purposes, as opposed to that tissue or those cell lineages (somatic tissue, or soma) producing the bodily structures and tissues used for functions other than reproduction.

Globin–a class of proteins most often associated with processes of oxygen or gas transport (e.g., hemoglobin or myoglobin).

Hemochromatosis—a pathological condition characterized by abnormal deposits of iron throughout the body; signs and symptoms include defects of the liver, glucose metabolism, and heart function.

Hemoglobin—a complex molecule that serves as the primary oxygen transport vehicle in vertebrates. It is composed of a single iron molecule surrounded by four globin molecules, two each of two different types (two alpha globins and two beta globins in normal adult humans).

Hemoglobinopathies—a collection of different, hereditary disorders of hemoglobin structure and/or function (e.g., thalassemia, sickle cell anemia).

Hemophilia–a hereditary disease distinguished by an abnormally long blood coagulation time. The important genes are recessive, and are found on the X-chromosome, making it X-linked; this means that it is most often seen in males, and most often transmitted to offspring by asymptomatic females.

Heterozygous--each normal cell in the body carries two copies of any given gene; if these two copies (alleles) are different one from another, or alternate forms of the same gene (e.g., blue v. brown eyes), then the individual is said to be heterozygous at that

locus. If they are identical, the individual is homozygous.

Homozygous--each normal cell in the body carries two copies of any given gene; if these two copies (alleles) are identical to each other (e.g., both coding for brown eyes) then the individual is said to be homozygous at that locus.

Huntington disease—''Huntington chorea"--a genetic disease that is not manifest until after birth (usually between the ages of 30 and 50) resulting in death due to progressive degeneration of specific brain tissues. The primary signs and symptoms are disorders of movement and dementia.

Hydrocephaly—a developmental defect marked by an unusual accumulation of spinal fluid in the ventricles of the brain. The malformation caused by this fluid buildup usually retards brain development, often resulting in mental retardation and, in severe cases, early death. The condition can now be treated if diagnosed soon after birth.

Hydroxyurea--an experimental drug used to promote expression of hemoglobin F genes (to replace defective Beta globin genes) in patients with thalassemia or sickle cell disease.

Hypercholesterolemia (familial)—a pathological condition of excess blood cholesterol that is inherited as an autosomal dominant trait,

Hyponatremia—a condition of low sodium concentrations in the blood.

Immune deficiencies—any of a number of conditions (e.g., adenosine deaminase deficiency, purine nucleoside phosphorylase deficiency, or AIDS) resulting from a failure or malfunction of the bodily defense mechanisms, or immune system.

Immunoglobins—a collection of complex protein molecules that play a vital role in the body's immune system.

Implantation—the process by which the fertilized egg (zygote) becomes attached to the wall of the uterus (endometrium) which then serves to nourish the embryo through growth and subsequent development.

in *utero* (in uterus) preferring to procedures that are performed or events that take place within the uterus .

in *vitro* (in glass) —meaning in the laboratory; in the test tube.

in vivo (in life) —meaning in the living, intact organism.

Klinefelter's syndrome—a chromosomal abnormality in human males. In contrast to the usual complement of sex chromosomes, one X and one Y (XY), Klinefelter males usually have two X's and one Y (XXY), although some have multiple Y's or more than two X's. Clinical symptoms are abnormal height, gonadal dysfunction (testicular atrophy; sterility), below average intelligence, and possibly some behavioral abnormalities (although this is still disputed by some).

Lesch-Nyhan syndrome-an X-linked recessive disorder characterised by compulsive self mutilation and other mental and behavioral symptoms. It is caused by a defect in the gene that produces a particular enzyme (hypoxanthine-guanine phosphoribosyl transferase) important in metabolism. In the absence of this enzyme large amounts of uric acid accumulate in the blood, leading to gout. The causal relationship to the behavioral disorder is not yet understood.

Linkage—the association, in inheritance, of different genes due to their physical proximity on chromosomes.

Lipid metabolism–the process by which lipid (fatty) molecules are broken down or synthesised in the body.

Liposome—a structure with a lipid membrane like that of a cell that can be filled with specific substances and then used as a delivery vehicle to transport those substances to the interior of a target cell by fusion with the cell's own membrane. It is one of several potential delivery vehicles for use in gene therapy.

Lysosomal storage diseases—lysosomes are intracellular organelles that contain enzymes capable of digesting proteins and some carbohydrates. Lysosomal storage diseases result from an accumulation of certain of these molecules caused by an insufficiency of a lysosomal enzyme. The symptoms and prognosis vary with the specific enzyme involved.

Marfan syndrome—arachnodacytly ("spider fingeredness")--a single gene defect of which the symptoms are abnormally long fingers and toes, abnormalities of the eye lenses and heart. (Abraham Lincoln is thought by some to have suffered from this disease).

Membrane fusion—a process by which the membranes (outer walls) of two cells merge, thus creating one daughter cell from two parents. In contrast to fertilization by gametes, membrane fusion describes the joining of somatic cells. One of the most productive results of membrane fusion technologies is the formation of hybridomas, wherein an antibody-producing white blood cell (leucocyte) is fused with a tumor cell to produce a daughter cell that can generate very large amounts of a specific antibody for use in diagnostic and therapeutic procedures (monoclinal antibodies).

Mendelian-–referring to a trait that is controlled by a single gene, and which therefore shows a simple pattern of inheritance (dominant or recessive). So

named because traits of this sort were first recognized by Gregor Mendel, the Austrian monk whose early researches laid the basis for modern genetics.

Messenger RNA (mRNA)—a ribonucleic acid molecule produced by transcribing a nucleotide base sequence from DNA into a complementary sequence of RNA. Messenger RNA molecules carry the instructions for assembling enzymes (protein molecules) from the chromosomes in the nucleus to the synthetic apparatus (ribosomes) in the cytoplasm, or cellular tissue outside the nucleus.

Metachromatic leukodystrophy (MLD)—several closely related disorders characterized by a degeneration of the protective sheath surrounding nerve cells (myelin) and an accumulation of certain metabolic compounds as a result of insufficient activity of the enzyme aryl sulfatase. Death is the result of progressive central nervous system degeneration accompanied by abnormalities of the peripheral nerves, kidney, and liver.

Metallothionein—a protein that binds metal ions. The promoter sequence that controls the production of metallothionein has been spliced to other genes and used to control their expression after gene transfer, as in, for example, the rat growth hormone transplanted into mice, resulting in "mighty" mice of larger than normal size.

Microinjection—the technique of introducing very small amounts of material (DNA or RNA molecules; enzymes; cytotoxic agents) into an intact cell through a microscopic needle penetrating the cell membrane.

Morula—the solid mass of cells resembling a mulberry ("morula" in Latin) formed by the cleavage of a zygote; the stage before blastocyst.

Mucopolysaccharidoses--a group of heritable diseases marked by defects in the metabolism of a class of molecule, the glycosaminoglycans (formerly called mucopolysaccharides). Symptoms usually include mental retardation (usually severe) and various skeletal abnormalities all accompanied by abnormal deposition of mucopolysaccharides in tissues or excretion in urine.

Muscular dystrophy (Duchenne type)—an X-linked recessive defect (therefore most affected individuals are male) of muscle metabolism that usually causes death by the age of twenty.

Multigenic disorder—(polygenic disorder)—a genetic defect resulting from the interaction of alleles of more than one gene. Although such disorders are heritable they depend on the simultaneous presence of several alleles and therefore the hereditary patterns are usually much more complicated than for simple, single-gene (Mendelian) traits, making prediction and diagnosis much more difficult.

Mutagen--any substance that can cause changes in the structure of hereditary nucleic acids (DNA, RNA) or the way the information they contain is transmitted to offspring.

Myopia (nearsightedness)--a defect in vision such that objects can be accurately resolved only when they are unusually close to the eyes. An autosomal dominant form is known, but many (perhaps most) cases are either non-Mendelian or complex in their mode of inheritance (i.e., polygenic, or involving variable expressivity or incomplete penetrance).

Neural tube defect-the neural tube is formed by the fusion of the neural folds, which are ridges of tissue that arise on either side of the primitive streak. The brain and spinal cord develop from the neural tube, and neural tube defects are any that affect their formation or development. Most such defects are developmental in origin; that is, though genetic factors may be involved these defects are more likely to be polygenic or complex rather than single gene, Mendelian traits.

Oligonucleotide–nucleic acid molecules formed by the joining of a small number of nucleotide bases (generally fewer than 10 or 20). A short sequence of DNA or RNA.

Oncogene--a gene of which one or more mutant forms is associated with cancer formation.

Ornithine carbamoyl transferase deficiency—(transcarbamylase deficiency)–an X-linked defect associated with a specific enzyme deficiency in the nitrogen cycle (transcarbamylase). Symptoms include chronic ammonia intoxication, mental deterioration, and liver failure.

Papilloma virus (Shope)—a DNA virus found in rabbits that is associated with elevated arginase activity levels in epithelial cells. (See argininemia).

Penetrance--refers to the frequency with which the effects of a gene (whether dominant or recessive) known to be present are seen in the individuals carrying it.

Peptide—a class of compounds formed by joining amino acids together by a chemical process that produces one molecule of water for each joining of one amino acid to another. Peptides are intermediate in size between amino acids and proteins.

Phage–see "bacteriophage."

Phenylketonuria (PKU)—an inborn error of metabolism, or genetic disease, caused by the inability to metabolize phenylalanine to tyrosine. The resulting accumulation of phenylalanine and derived products causes mental retardation. The disease is due to a defective enzyme (phenylalanine hydroxylase), and the symptoms can be treated and the condition ameliorated with a diet that eliminates phenylalanine. The disease can be diagnosed at birth by a sim-

ple test that detects the characteristic elevated levels of phenylpyruvic acid (a phenylalanine derivative) in the urine.

Plasmid—a circular piece of DNA found in the cytoplasm, outside the nucleus. Replication and segregation of plasmids to daughter cells is independent of the chromosomes, and plasmid transmission from parent to offspring is almost exclusively matrilineal (from mother to offspring), because while plasmids are common in ova they are generally absent from that portion of the sperm that fuses with the ovum to form a zygote.

PNP deficiency—an autosomal recessive disorder of immunity caused by deficiency of the enzyme purine nucleoside phosphorylase.

Polycystic kidney disease—a hereditary disease (single gene dominant) in which a progressive deterioration of kidney function is associated with the development of large numbers of cysts.

Polygenic—referring to a trait or characteristic that is controlled not by one gene but rather by two or more acting in concert.

Polymerization—the process of joining molecular subunits (e.g., nucleotide base pairs) together in sequence to form a larger molecule (e.g., a polynucleotide). primitive streak—the first visible sign of differentiation **in the developing embryo. It is a darkened longitudinal stripe that forms at the caudal (tailward) end of the embryo, and is composed of a layer of ectodermal cells (which develop into skin and nervous tissue) and it marks the future location of the longitudinal exis of the embryo.**

probes—molecules that make it possible to seek out and identify specific cellular features (see DNA probes).

Promoter—a region of a DNA molecule found in front of a gene (as the DNA molecule is "read" by the proper enzymes) that controls the expression of the gene.

Protoplasm (first formed)—a single cell or a mass of protoplasm (the substance of which cells are formed). The term usually refers to a bacterial cell or to an individual plant cell from which the cell wall has been removed preparatory to cell-fusion experiments.

Pyridoxine responsive hemocystinuria—a condition of excess cystine in the blood that can be treated with the drug pyridoxine.

Recessive—(contrast with *Dominant)* referring to an allele of a gene that will not be seen in the phenotype of the organism carrying it unless it is present in two copies (i.e., on both chromosomes), or homozygous. If present in only one copy, or heterozygous, its presence will be masked. (See *Carrier). X-***linked traits generally act as if they were recessive in females and dominant in males.**

Recombinant DNA (rDNA)—referring to DNA molecules that have been assembled with the use of restriction enzymes, usually (but not always) by splicing together fragments from different species.

Restriction enzyme—an enzyme that has the ability to recognize a specific nucleotide sequence in a nucleic acid (ranging from four to twelve base pairs in length) and cut, or cleave, the nucleic acid at the point. So called because, occurring naturally in bacteria, they recognize foreign nucleic acid (e.g. the DNA of a bacterial virus as it begins to infect and destroy its host) and destroy it, thus restricting the ability of the virus to prey upon certain potential host strains. Over four hundred different restriction enzymes are known, recognizing a great variety of different nucleotide base sequences. This has made possible the cutting and splicing together of nucleic acid within and between different organisms and species.

Ribosome—a cellular organelle which is the site of messenger RNA translation, the process of reading the instructions in an mRNA molecule and using them as the guide to constructing the specified protein. Ribosomes are composed of both RNA and protein, and they spontaneously assemble from the necessary constituents present in the cell.

RNA (Ribonucleic acid)—a polynucleotide consisting of a backbone of alternating phosphate and sugar (ribose) molecules to which are attached the nucleotide bases adenine (A), thymine (T), guanine (G) and uracil (U, which replaces the cytosine, C, of DNA). There are several classes of RNA that serve different purposes, including messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). (See Technical Notes.)

Sickle cell disease (anemia)—a hereditary hemoglobinopathy caused by the presence of a defective beta hemoglobin chain. Patients with sickle cell disease have red blood cells that tend to deform into a sickle-like shape when the abnormal hemoglobin crystallizes. The specific defect is caused by an abnormal gene resulting in the replacement of the usual amino acid, glutamic acid, with valine, in the sixth amino acid position in the beta-hemoglobin molecule. This alters the resulting beta globin molecule in such a way as to increase its propensity to crystallize, thus rupturing the red blood cell and causing the cells to lodge in small blood vessels.

Sickle cell trait—refers to a person who is heterozygous for the gene producing the abnormal form of the beta hemoglobin chain. People carrying the sickle cell gene in heterozygous form (carriers) are

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usually asymptomatic, and thus not afflicted by the disease. Under some conditions of extreme exertion that reduce the concentration of oxygen in the blood a small amount of sickling of red blood cells may be detected, but usually not enough to bring on any of the pathological conditions of the disease. The mutation is found with high frequency in some populations subject to malarial infections, such as African blacks. The defective gene is thought to be maintained in the population because it confers increased resistance to malaria upon heterozygotes.

Single gene disorder (Mendelian disorder)--a genetic disease caused by a single gene that shows a simple pattern of inheritance (e.g., dominant or recessive, autosomal, or X-linked).

Somatic—referring to body tissues apart from reproductive (germinal) tissues.

Tay-Sachs disease—an autosomal recessive genetic defect resulting in developmental retardation, paralysis, dementia, and blindness followed by death, usually before the end of the third year of life. The defective gene codes for hexosaminidase A, an enzyme that degrades certain chemicals in the brain. Symptoms are caused by an accumulation of cerebral gangliosides, fatty acid, and sugar molecules found in the brain and nervous tissue. The gene is found in highest frequency among Ashkenazic Jews of Eastern European origin.

Tetramer--a complex molecule consisting of four major portions (moieties) joined together in some reversible, non-structural manner (e.g., hemoglobin, in which two alpha chains and two beta chains are joined by electromagnetic attractions).

Thalassemia--any of several heritable hemoglobinopathies resulting from defective genes causing deletions or other alterations of different hemoglobin molecules.

Transcription—the process by which a complementary messenger RNA (mRNA) molecule is formed from a single stranded DNA template. The result of the process is that the information contained in DNA is transferred to mRNA which is then used as a template to direct the construction of protein molecules that function in cellular metabolism.

Transferrin—a protein molecule that carries iron in blood plasma. A number of different, genetically coded molecules are known.

tRNA (transfer RNA)--specialized RNA molecules that function to bring specific amino acids from the cellular environment to ribosomes that are translating mRNA into proteins (constructing proteins according to the information encoded in the parent DNA template from which the mRNA was copied).

Translation—the process of decoding the information in an mRNA molecule and using it to direct the construction of protein molecules specified in the messenger RNA.

Transposable elements—a class of DNA molecules capable of insertion into the chromosomes of the host organism at any or several of numerous positions, and of moving from one position to another. Speculation on the origin of these molecules suggests that they may be derived from virus-like ancestors. They have been called "parasitic" DNA.

Ultrasound—high frequency sound waves that can be focused and used to picture tissues, organs, structures, or tumors within the body. Ultrasound is particularly useful for in *utero* examinations of the fetus. It is often used to locate the fetus and the placenta prior to such procedures as amniocentesis or chorionic villus biopsy.

Urea-cycle defects—the urea cycle is the metabolic pathway in the body that moves nitrogen from one source to another, and takes it out of and puts it into the body chemistry when and where needed. Each different step is mediated by one or more enzymes, all of which are genetically controlled and which can, under the influence of abnormal genes, lead to different genetic diseases (inborn errors of metabolism) that are collectively known as urea-cycle defects.

Wernicke-Korsakoff encephalopathy—a genetic disease (probably autosomal recessive) of oxalate metabolism caused by a defective transketolase enzyme. It seems to become clinically important only when the diet is deficient in thiamine, can be exacerbated by alcohol and treated with vitamin B 1 supplements.

Wilson disease--an autosomal recessive disease of copper metabolism in which various abnormalities of the liver are accompanied by different neurological symptoms.

X-linked—referring to traits found on the X chromosome. X-linked recessive traits are seen far more often in males, who have only one X chromosome, than in females, who have two.

Zygote—a fertilised egg; a product of the fusion of sperm and egg.

Appendix F

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