

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

JANUARY 27, 1986

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RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING<sup>1</sup>

January 27, 1986

The Recombinant DNA Advisory Committee (RAC) was convened for its thirty-fourth meeting at 9:00 a.m. on January 27, 1986, in Building 31, Conference Room 10, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892. Mr. Robert Mitchell (Chair), Attorney at Law in California, presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Barbara Bowman	Wolfgang Joklik	Jeffrey Roberts
Royston Clowes	Edward Korwek	Frances Sharples
Mitchell Cohen	J. Robert MacNaughton	Anne Vidaver
Bernard Davis	Mark Mills	LeRoy Walters
Charles Epstein	Robert Mitchell	Anne Witherby
Susan Gottesman	Gerald Musgrave	William J. Gartland, Jr.
Irving Johnson	Thomas Pirone	(Executive Secretary)

A committee roster is attached (Attachment I).

Ad hoc consultants:

David Friedman, University of Michigan  
David Martin, Genentech, Inc., South San Francisco  
Dusty Miller, Fred Hutchinson Cancer Research Center  
Robertson Parkman, Children's Hospital of Los Angeles  
Mark Saqinor, Metabolic Research Medical Group, Inc., Los Angeles

<sup>1</sup>The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Non-voting agency representatives:

Joseph Autry, Department of Health and Human Services  
 Howard M. Berman, Veterans Administration  
 Bernard Greifer, Department of Commerce  
 Edwin Shykind, Department of Commerce  
 Sue A. Tolin, Department of Agriculture  
 Morris A. Levin, Environmental Protection Agency  
 Herman W. Lewis, National Science Foundation

Liaison representatives:

Daniel P. Jones, National Endowment for the Humanities

National Institutes of Health staff:

W. French Anderson, NHLBI  
 Stanley Barban, NIAID  
 Irving Delappe, NIAID  
 Rosalind Gray, OD  
 Sandra Hecker, NIGMS  
 Philip Kantoff, NHLBI  
 Stefan Karlsson, NHLBI  
 Becky Lawson, NIAID  
 Rachel Levinson, OD  
 Jean McHachlin, NHLBI  
 Elizabeth Milewski, NIAID  
 Robert T. Simpson, NIADDK  
 Virginia Start-Vancs, NHLBI  
 Bernard Talbot, NIAID  
 Jamie Zweibel, NHLBI

Others:

C. Blakey, Federation of American Societies for Experimental Biology  
 Chia Ting Chen, Department of Labor  
 Barbara Culliton, Science Magazine  
 Theodore J. DeLoggio, Pennwalt Corporation  
 Joel Dalrymple, Department of Defense  
 Dominic D. Diascro, Pennwalt Corporation  
 Peter Farnham, American Society for Biological Chemists  
 Ralph M. Faust, Georgetown University  
 Gershon W. Fishbein, Genetic Engineering Letter  
 Jeffrey Fox, American Society for Microbiology  
 Keith Haglund, International Medical Tribune  
 Iclal Hartman, Lexington Biohazard Committee  
 Susan Jenks, Medical World News  
 Dorothy Jessup, Department of Agriculture

Alwyn Johnson  
Roger Johnson, Genetic Engineering News  
Attila T. Kadar, Food and Drug Administration  
John H. Keene, Abbott Laboratories  
Rihito Kimura, Kennedy Institute  
Karen Kocher, Commerce Clearinghouse  
Jean Marx, Science Magazine  
Jeff McCullough, University of Minnesota  
Michael Norton, British Embassy  
Greg Pearson, Blue Sheet  
Reginald Rhein, McGraw-Hill World News  
Jane Rissler, Environmental Protection Agency  
Marvin Rogul, The Rogul Group  
Harold M. Schmeck, New York Times  
Mark C. Segal, Environmental Protection Agency  
Brian T. Sheehan, Cooper Biomedical  
Gary Spies, Genetics Institute  
Robert Steinbrook, Los Angeles Times  
Clarence E. Styron, Monsanto Company  
Joseph Van Houten, Schering-Plough  
William J. Walsh, National Academy of Sciences  
Stephanie Zobrist, Embassy of Switzerland

## I. CALL TO ORDER AND OPENING REMARKS

Mr. Mitchell, Chair, called the January 27, 1986, meeting of the Recombinant DNA Advisory Committee (RAC) to order. He said this public meeting had been formally announced in the December 20, 1985, Federal Register (50 FR 52176). No written comments have been received on the major actions announced in that Federal Register. Mr. Mitchell said comments can be submitted after the meeting and used to assist the Director, NIH, in arriving at a decision.

Dr. Gartland informed Mr. Mitchell that a quorum was present for the January 27, 1986, meeting. Mr. Mitchell said he would recognize speakers in the following order: primary reviewers; other RAC members; ad hoc consultants; non-voting representatives to the RAC; RAC's administrative staff; members of the public who submitted written documents or comments; and finally other members of the public who wish to comment.

Mr. Mitchell introduced the newly appointed members of the RAC: Dr. Charles Epstein of the University of California, San Francisco; Dr. Edward Korwek of the law firm of Keller and Heckman of Washington, D.C.; Dr. Gerald Musgrave, President of Economics America, Inc., of Ann Arbor, Michigan; Dr. Paul Neiman of the Fred Hutchinson Cancer Research Center in Seattle, Washington; Dr. Jeffrey Roberts of Cornell University in Ithaca, New York; and Mr. J. Robert McNaughton an economist and administrator from Bethesda, Maryland.

Mr. Mitchell then introduced the ad hoc consultants for this meeting: Dr. David Friedman of the University of Michigan; Dr. Mark Saginor of the Metabolic Research Medical Group, Inc., of Los Angeles, California; Dr. David Martin of Genentech Inc., of South San Francisco, California; Dr. A. Dusty Miller of the Fred Hutchinson Cancer Research Center in Seattle, Washington; and Dr. Robertson Parkman of Childrens Hospital of Los Angeles, California.

## II. MINUTES OF THE SEPTEMBER 23, 1985, RAC MEETING

Mr. Mitchell called on Ms. Witherby to review the minutes (tab 1256) of the September 23, 1985, RAC meeting. Ms. Witherby said the minutes appear to be accurate. Ms. Witherby and Dr. Walters each pointed out a typographical error.

Dr. Epstein moved approval of the minutes as corrected. Dr. Gottesman seconded the motion. The minutes were unanimously approved.

## III. PROPOSAL TO MODIFY APPENDIX J

Mr. Mitchell called on Dr. Saginor to begin discussion of the proposal (tabs 1250/IV, 1253) to modify Appendix J of the NIH Guidelines.

Dr. Saginor said current Appendix J of the NIH Guidelines for Research Involving Recombinant DNA Molecules describes the Federal Interagency Advisory Committee on Recombinant DNA Research and lists those departments and agencies which had representation on this committee as of December 1980. This committee has not met since 1980. Recently another interagency committee, the Biotechnology Science Coordinating Committee (BSCC), has been established to provide for interagency science policy coordination and guidance and for exchange of information. The formation of this committee was announced in a Federal Register notice on November 14, 1985.

Dr. Talbot, Deputy Director of the National Institute of Allergy and Infectious Diseases, in a memorandum dated November 25, 1985, suggested Appendix J of the NIH Guidelines be modified to reflect the current situation; current Appendix J would be deleted and the following language substituted:

"Appendix J. Biotechnology Science Coordinating Committee

"The following excerpts from its charter (signed October 30, 1985) describe the Biotechnology Science Coordinating Committee:

"Purpose

"The Domestic Policy Working Group on Biotechnology has determined that in the area of biotechnology with its rapid growth of scientific discovery, scientific issues of interagency concern will arise frequently and need to be communicated among the various agencies involved with reviews of biotechnology applications. The Federal Coordinating Council for Science, Engineering, and Technology (FCCSET) established by 42 U.S.C. 6651 is an interagency science committee chaired by the Director of the Office of Science and Technology Policy with the mission of coordinating science activities affecting more than one agency. Committees may be established under FCCSET for addressing particular science issues. Thus, the Biotechnology Science Coordinating Committee (BSCC) is established to provide formally an opportunity for interagency science policy coordination and guidance and for the exchange of information regarding the scientific aspects of biotechnology applications submitted to Federal research and regulatory agencies for approval.

"Functions

The BSCC will coordinate interagency review of scientific issues related to the assessment and approval of biotechnology research applications and biotechnology product applications and postmarketing surveillance when they involve the use of recombinant RNA, recombinant DNA, cell fusion or similar techniques. The BSCC will:

- "(a) Serve as a coordinating forum for addressing scientific problems, sharing information, and developing consensus;

- "(b) Promote consistency in the development of Federal agencies' review procedures and assessments;
- "(c) Facilitate continuing cooperation among Federal agencies on emerging scientific issues; and
- "(d) Identify gaps in scientific knowledge.

"Authority

"To accomplish these functions the BSCC is authorized to:

- "(a) Receive documentation from agencies necessary for the performance of its function;
- "(b) Conduct analyses of broad scientific issues that extend beyond those of any one agency;
- "(c) Develop generic scientific recommendations that can be applied to similar, recurring applications;
- "(d) Convene workshops, symposia, and generic research projects related to scientific issues in biotechnology; and
- "(e) Hold periodic public meetings.

"Members and Chairman

"The BSCC includes the following initial members:

"Department of Agriculture

Assistant Secretary for Marketing and Inspection Services  
Assistant Secretary for Science and Education

"Department of Health and Human Services

Commissioner, Food and Drug Administration  
Director, National Institutes of Health

"Environmental Protection Agency

Assistant Administrator for Pesticides and Toxic Substances  
Assistant Administrator for Research and Development

"National Science Foundation

Assistant Director for Biological, Behavioral and Social Sciences

"The BSCC is chaired by the Assistant Director for Biological, Behavioral and Social Sciences of the National Science Foundation and the Director of the National Institutes of Health on a rotating basis.

"Administrative Provisions

- "(a) The BSCC will report to the FCCSET through the Chair.
- "(b) Meetings of the BSCC shall be held periodically. Some public meetings will be held.
- "(c) Confidential business information and proprietary information shall be protected under the confidentiality requirements of each member agency.
- "(d) Subcommittees and working groups, with participation not restricted to BSCC members or full-time Federal employees, may be formed to assist the BSCC in its work.
- "(e) All BSCC members will be full-time Federal employees whose compensation, reimbursement for travel expenses and other costs shall be borne by their respective agencies.
- "(f) Each member of the BSCC shall provide such agency support and resources as may be available and necessary for the operation of the BSCC including undertaking special studies as come within the functions assigned herein.
- "(g) An Office of Science and Technology Policy staff member will serve as BSCC Executive Secretary."

Dr. Walters said he favored this modification of Appendix J. However, he wished to note that an "asymmetry" exists between RAC's purview and the purview of the BSCC. The RAC deals with recombinant DNA and RNA derived from recombinant DNA. The BSCC addresses recombinant DNA, recombinant RNA and cell fusion.

Dr. Johnson pointed out that some responsibilities of the BSCC will overlap RAC responsibilities. The BSCC has stated its intention to be involved in scientific issues related to assessment and approval of biotechnology research applications; these issues in regard to recombinant DNA research have traditionally been the purview of the RAC.

Dr. Sharples said the current Appendix J of the NIH Guidelines indicates that the members of the Federal Interagency Advisory Committee had endorsed and agreed to abide by the NIH Guidelines. She asked how the creation of the BSCC and modification of Appendix J would affect the agreement to abide by the NIH Guidelines. Dr. Talbot said this proposed modification should have no effect on the status of agency agreements to abide by the NIH Guidelines.

Dr. Gottesman moved acceptance of the proposal to modify Appendix J of the NIH Guidelines. Dr. Cohen seconded the motion. By a vote of seventeen in favor, none opposed, and no abstentions, the RAC approved the motion.

#### IV. PROPOSAL TO MODIFY APPENDIX C

Dr. Friedman began the discussion of the proposal (tabs 1250/II, 1254, 1259) to add a new section to Appendix C of the NIH Guidelines. This modification would exempt certain types of experiments involving extrachromosomal elements and gram positive bacteria.

The proposal originated with the RAC Working Group on Gram Positive Bacteria which was convened in response to a request by Drs. Richard Novick and Joan Polak of the Public Health Research Institute of the City of New York. Drs. Novick and Polak requested that a comprehensive exchanger list be established for the gram-positive Eubacteriales.

At the December 2, 1985, meeting of the Working Group on Gram Positive Bacteria it was noted that Section III-D-4 of the NIH Guidelines states that certain classes of recombinant DNA molecules are exempt from the NIH Guidelines; organisms exempt under Section III-D-4 are listed in Appendix A. Dr. Friedman described the philosophical basis of Appendix A. In evaluating organisms for inclusion on an Appendix A list, an estimate is made of the probability that the combination of genes might occur in nature. If the combination could occur in nature, there should be no special hazard in creating such an organism by recombinant DNA techniques; and the combination of organisms can then be exempted from the NIH Guidelines.

Genes are exchanged between bacteria in nature by transduction, transformation, or conjugation and evidence showing such exchange is the basis for inclusion in Appendix A. Four categories of conjugal genetic exchange are considered in evaluating the probability that the combination might occur in nature: (1) organisms which exchange chromosomal genetic information which becomes stably integrated into the host chromosome; (2) organisms which exchange chromosomal information that is not necessarily integrated into the chromosome of the recipient; (3) organisms which show evidence of a plausible mechanism for exchange; and (4) organisms which can receive or donate broad host range plasmids.

In 1978, RAC accepted evidence of chromosomal exchange and plasmid mobilization of chromosomal genes between organisms as an indication that no "novel" organisms would be created through laboratory combination of the genes of these organisms. Later, information accumulated in gram-negative bacteria demonstrated that chromosomal gene exchange occurred at some frequency when plasmid exchange occurred and RAC accepted that plasmid exchange in gram-negative bacteria could result in transfer of chromosomal genes.

However, exchange of DNA in gram-positive bacteria does not appear to follow the model observed in gram-negative bacteria. F' episomes are not known in gram-positive bacteria and chromosomal gene transfer by this type of conjugation has not been described in these organisms. Good and abundant evidence demonstrates the transfer of broad host-range plasmids in the gram-positive Eubacteriales. However, little or no evidence exists for exchange of chromosomal DNA by this mechanism although most

experts feel chromosomal exchange occurs by this mechanism in gram-positive bacteria in nature.

On the basis of the available data, the Working Group on Gram Positive Bacteria suggested that a new section be added to Appendix C. That new section would be entitled Appendix C-V, Extrachromosomal Elements of Gram Positive Organisms. The current Appendix C-V, Footnotes and References of Appendix C, would be renumbered Appendix C-VI under the proposal. Proposed Appendix C-V would read as follows:

"Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from the NIH Guidelines.

"Bacillus subtilis  
Bacillus pumilus  
Bacillus licheniformis  
Bacillus thuringiensis  
Bacillus cereus  
Bacillus amyloliquefaciens  
Bacillus brevis  
Bacillus natto  
Bacillus niger  
Bacillus atterimus  
Bacillus amylosacchariticus  
Bacillus anthracis  
Bacillus globigii  
Bacillus megaterium  
Staphylococcus aureus  
Staphylococcus epidermidis  
Staphylococcus carnosus  
Clostridium acetobutylicum  
Pediococcus damnosus  
Pediococcus pentosaceus  
Pediococcus acidilactici  
Listeria grayi  
Listeria murrayi  
Listeria monocytogenes  
Streptococcus pyogenes  
Streptococcus agalactiae  
Streptococcus sanguis  
Streptococcus salivarius  
Streptococcus cremoris  
Streptococcus pneumoniae  
Streptococcus avium  
Streptococcus faecalis  
Streptococcus arginosus  
Streptococcus sobrinus  
Streptococcus lactis  
Streptococcus mutans

Streptococcus equisimilis  
 Streptococcus thermophilus  
 Streptococcus milleri  
 Streptococcus durans  
 Streptococcus mitior  
 Streptococcus ferus

"Exceptions.

"Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

"Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III-B-5).

"Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F)."

Dr. Milewski informed the committee that the organism Lactobacillus casei had inadvertently been deleted by typographical error from the list published in the Federal Register.

Dr. Friedman said the Working Group on Gram Positive Bacteria also suggested the first paragraph of Appendix C-IV, Experiments Involving Bacillus subtilis Host-Vector Systems, be modified. That paragraph currently reads as follows:

"Any asporogenic Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than  $10^{-7}$  can be used for cloning DNA with the exception of those experiments listed below. Indigenous Bacillus plasmids and phages whose host-range does not include Bacillus cereus or Bacillus anthracis may be used as vectors."

The second sentence of that paragraph would be deleted and the paragraph would read as follows:

"Any asporogenic Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than  $10^{-7}$  can be used for cloning DNA with the exception of those experiments listed below."

Dr. Friedman said most experiments involving gram-positive organisms may currently be performed under Section III-C of the NIH Guidelines. Section III-C specifies Biosafety Level (BL) 1 containment conditions and requires notification of the Institutional Biosafety Committee (IBC) at the time of initiation of the experiment. Dr. Friedman said the working group hoped the proposed modification would simplify review procedures for protocols involving broad host-range plasmids and organisms on the proposed list.

Dr. Clowes said he was sympathetic to the view that, if good evidence of exchange of extrachromosomal elements exists, chromosomal gene transfer almost surely takes place. He would have liked to have seen a broader exemption; nonetheless, he supported the working group proposal.

Dr. Davis said he felt some concern because the recommendation is based on a listing of species. Speciation in bacteria is quite arbitrary in comparison to speciation in higher organisms to which an interfertility criterion can be applied. He wondered how future changes in the definition of a species would affect the working group recommendation. Would newly named or renamed species become "non-exempt" until the NIH modified the list?

Dr. Davis favored the drafting of language which states general criteria rather than listing specific organisms. He moved that language such as the following be added to the working group proposal: "any gram positive organism that shall be demonstrated to meet the criteria of genetic exchange accepted by the committee for this list of organisms shall also be exempt." He said his proposal was an attempt to avoid "setting up a situation that will provide maximal protection at the cost of unnecessary delays." Dr. Johnson supported Dr. Davis' proposal.

Dr. Clowes also supported Dr. Davis' suggestion; he felt organisms could be caught in a bureaucratic tangle if species were renamed and generic language describing the criteria for inclusion on the list did not exist.

Dr. Walters said a specific listing of organisms offers an advantage: investigators would immediately know by reading the list whether an organism met the specified criteria. If Dr. Davis' additional wording were added, investigators would presumably need to submit data that their organism met the criteria, and some group would have to review the data and make a decision whether the criteria had been fulfilled.

Dr. Gottesman pointed out that most experiments involving gram-positive Eubacteriales are currently covered by Section III-C of the NIH Guidelines. Since these experiments can be performed under B11 containment with notification to the IBC, experiments involving these organisms are possible under relatively simple procedures. She said she did not oppose generic language but it should be fully discussed before it is added to the NIH Guidelines. In addition, the language should clearly indicate who will decide that the criteria for exemption have been met in specific cases; shall it be the principal investigator, the IBC, or ORDA?

Dr. Talbot suggested that RAC act on the proposal submitted by the working group since that language had been published in the Federal Register. A generic statement could subsequently be developed and published for public comment in the Federal Register prior to a future RAC meeting.

Dr. Davis withdrew his motion; he recommended the working group consider developing generic language for future consideration.

Dr. Friedman moved acceptance of the motion as it appeared in the Federal Register with the addition of Lactobacillus casei to the list. Dr. Clowes seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, RAC recommended the motion.

V. PROPOSED REVISION OF APPENDIX C-I

Mr. Mitchell asked Dr. Friedman to describe the proposal (tabs 1250/III, 1251) to modify Appendix C-I, Recombinant DNAs in Tissue Culture, of Appendix C, Exemptions Under Section III-D-5.

Dr. Friedman said Dr. Robert Simpson, Chair of the NIH IBC, requested a modification of the NIH Guidelines to exempt some experiments in tissue culture involving viral sequences. Currently experiments in which eukaryotic viral sequences are propagated in tissue culture cells must be registered with the IBC. In requesting this modification, Dr. Simpson pointed out that many experiments in tissue culture involve the use of "shuttle" vectors containing small amounts of eukaryotic viral DNA and bacterial plasmid sequences. Examples of the types of viral sequences utilized in these constructs are the SV40 origin of replication, splice junction, polyadenylation signal, and the herpes thymidylate kinase gene (TK).

Dr. Friedman said the NIH IBC contended that since such shuttle vectors are not capable of generating infectious virus the registration requirement is not necessary. Dr. Simpson suggested Appendix C-I be modified to read as follows:

"Appendix C-I. Recombinant DNAs in Tissue Culture. Recombinant DNA molecules that are propagated and maintained in cells in tissue culture are exempt from the NIH Guidelines with the exceptions listed below.

Exceptions.

"Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

"Experiments involving DNA from Class 3, 4, or 5 organisms (1) or cells known to be infected with these agents.

"Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

"Experiments involving DNA molecules which contain more than two-thirds of a primate retroviral genome."

Dr. Gottesman said the issue of how the NIH Guidelines should regard small fragments of viral genomes in tissue culture was discussed by the RAC Working Group on Viruses at its November 12, 1985, meeting. She said her understanding of the working group discussion was that if an infectious agent is not involved, tissue culture cells are essentially completely contained since there is no mechanism by which recombinant DNA could move to other systems. For this reason, the NIH Guidelines exempt experiments in tissue culture when no viral DNA is involved. As the recently developed non-infectious shuttle vectors containing small pieces of DNA of viral origin do not provide a mechanism for dissemination of recombinant DNA from tissue culture, it is logical to exempt these types of vector constructs

from the NIH Guidelines when they are used in tissue culture. The challenge is to develop generic language which would exempt such constructs but not exempt from IBC review experiments involving infectious agents. Past approaches to similar issues specified some portion of the genome as "defective" and placed defective fragments under less stringent restraints than nondefective particles. The Working Group on Viruses discussed this issue but did not reach agreement on what percentage of a viral genome could be considered a "defective" fragment.

Dr. Joklik agreed with Dr. Gottesman's summary of the working group discussion. He said his recollection of the intent of the working group was to exempt viral regulatory sequences and genes such as the TK gene so that IBC registration would not be required for commonly used shuttle vectors.

Dr. Miller asked if amphotropic oncogenic retroviruses would be exempt under the language proposed by Dr. Simpson. He did not think it appropriate to exempt such viruses. Dr. Joklik replied that those types of viruses would be exempt under Dr. Simpson's proposed language.

Dr. Gottesman asked if RAC agreed with the concept of exempting in tissue culture small non-infectious pieces of viral DNA, and there was agreement. She asked for comments on language exempting fragments of less than 3 kilobases (Kb). Dr. Joklik said 3 Kbs is somewhat less than half of two types of genomes that might be of concern, retroviruses and papovaviruses; a 3 Kb limit would exempt all origins of replication, polyadenylation signals, splicing signals, etc.

Dr. Talbot said this could be accomplished by changing the fourth "exception" in Dr. Simpson's proposal to read:

"Experiments involving DNA molecules which contain more than 3 Kb of a viral genome."

Dr. Gottesman expressed concern that a statement under "exceptions" might not be sufficiently obvious.

Dr. Walters asked whether this proposal would cover both RNA and DNA viruses. Dr. Gottesman replied that it would.

Dr. Miller pointed out that a shuttle vector construct might contain origins of replication, polyadenylation signals, and other regulatory genes of viral origin. The total amount of viral DNA in one shuttle construct might exceed 3 Kb but the vector would be noninfectious. He asked if it would be possible to modify the proposed language to accommodate such constructs.

Dr. Barban pointed out that the TK gene, a commonly used marker in many vectors, is 3.4 Kb. Vectors containing the TK gene would not be exempt under the proposed language.

Dr. Gottesman moved that Appendix C-I be modified to read as follows:

"Recombinant DNA molecules containing less than one-half of a eukaryotic viral genome (all viruses from a single Family being considered identical) and that are propagated and maintained in cells in tissue culture are exempt from the NIH Guidelines...."

Dr. Epstein suggested the word "and" be deleted from the phrase "and that are propagated." Dr. Gottesman agreed. Dr. Joklik seconded the modified motion.

Dr. Walters asked why the clause "all viruses from a single Family being considered identical" was included in the motion. Dr. Gottesman explained that the clause precludes the exemption of experiments involving closely related viruses where the total of their combined DNA exceeds one-half of the genome since infectious particles might be regenerated from such experiments. Similar text already exists in the NIH Guidelines in the Note in Section III-B-3 and its two explanatory footnotes. These footnotes, appropriately modified, should also be now added to Appendix C-I.

Mr. Mitchell called the question. By a vote a seventeen in favor, none opposed, and no abstentions, the RAC accepted the motion.

Later in the meeting, Dr. Miller suggested that the word "any" should be substituted for the article "a" in the phrase "one half of a eukaryotic viral genome."

Dr. Gottesman moved that RAC reconsider the earlier motion. Dr. Cohen seconded the motion. The RAC voted to reconsider the earlier motion by a vote of seventeen in favor, none opposed, and no abstentions.

Dr. Gottesman then moved to substitute the word "any" for the article "a" in the phrase "one half of a eukaryotic viral genome." Dr. Walters seconded the motion. By a vote of seventeen in favor, none opposed, and no abstentions the motion was carried.

#### VI. PROPOSED MODIFICATIONS OF THE GUIDELINES TO REFER TO RNA

Dr. Gottesman said this proposal (tabs 1250/I, 1252) addresses the fact that the current NIH Guidelines do not explicitly refer to RNA. As the RNA-containing retroviruses are increasingly being used as vectors, it makes sense for the NIH Guidelines to refer explicitly to RNA. A RAC working group, therefore, developed a proposal to modify Section III-A-4, Section III-B-3, and Section III-B-4-a of the NIH Guidelines.

Section III-A-4 of Section III-A, Experiments that Require RAC Review and NIH and IBC Approval Before Initiation, currently reads as follows:

"III-A-4. Deliberate transfer of recombinant DNA or DNA derived from recombinant DNA into human subjects (21). The requirement for RAC review should not be considered to preempt any other required review of experiments with human subjects. Institutional Review Board (IRB) review of the proposal should be completed before submission to NIH."

Under the working group proposal, the first sentence of Section III-A-4 would be modified to read as follows:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects (21)."

Section III-B-3 of Section III-B, Experiments that Require IBC Approval Before Initiation, currently reads as follows:

"III-B-3. Experiments Involving the Use of Infectious Animal or Plant Viruses or Defective Animal or Plant Viruses in the Presence of Helper Virus in Tissue Culture Systems.

"Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions which permit a productive infection. In such cases, serious consideration should be given to raising the physical containment by at least one level.

"Note.--Recombinant DNA molecules which contain less than two-thirds of the genome of any eukaryotic virus (all virus from a single Family (17) being considered identical (19)) may be considered defective and can be used in the absence of helper under the conditions specified in Section III-C."

Under the working group proposal, the title of Section III-B-3 would be modified to read as follows:

"III-B-3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems.

The note would be modified to read as follows:

"Note.--Recombinant DNA molecules or RNA molecules derived therefrom which contain less than two-thirds of the genome of any eukaryotic virus (all virus from a single Family (17) being considered identical (19)) may be considered defective and can be used in the absence of helper under the conditions specified in Section III-C."

Section III-B-4-a of Section III-B, Experiments that Require IBC Approval Before Initiation, currently reads as follows:

"III-B-4-a. DNA from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study (2). It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with Class 5 agents (18, 20)."

Under the working group proposal, the first sentence of Section III-B-4-a would be modified to read as follows:

"III-B-4-a. Recombinant DNA or RNA molecules derived therefrom from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study (2)."

Dr. Gottesman said this proposal was evaluated by the Working Group on Viruses at its November 12, 1985, meeting, and supported by that group.

Dr. Korwek asked if this proposal referred specifically to RNA derived from DNA or to recombinant RNA. He noted that the RAC charter referred to recombinant DNA and would have to be revised if RAC's jurisdiction was to be extended to recombinant RNA. Dr. Gottesman replied that the proposal referred to RNA derived from recombinant DNA. She said that while the description of the proposal in the Federal Register announcement refers to "recombinant RNA," the language which would be added to the NIH Guidelines does not include the term "recombinant RNA."

Dr. Joklik said there is currently no such thing as "recombinant RNA." There are no known restriction nucleases which cleave RNA and no known ways to join RNA segments outside living cells.

Dr. Clowes suggested an explanation of the intent of the proposal would be useful to IBCs. He also suggested that the phrase "e.g., retroviral vectors" be added to the proposed language to indicate the purpose of the modifications. Dr. Davis disagreed with the second suggestion.

Dr. Miller supported the proposal as published in the Federal Register.

Dr. Korwek asked if the current phrase in Section III-A-4, "DNA derived from recombinant DNA," was not superfluous. Dr. Gottesman said that language had been inserted in this section of the NIH Guidelines to specifically cover segments of DNA even if they were treated with restriction nucleases to separate them from other segments of DNA to which they had been previously joined outside living cells.

Dr. Gottesman moved that RAC accept the proposed modifications of Sections III-A-4, III-B-3, and III-B-4-a as announced in the December 20, 1985, Federal Register with the suggestion that the Office of Recombinant DNA Activities (ORDA) transmit a memorandum to IBCs explaining the intent of this proposal. Dr. Epstein seconded the motion.

Mr. Mitchell called the question. The motion was carried by a vote of fifteen in favor, none opposed, and one abstention.

## VII. REPORT OF THE WORKING GROUP ON VIRUSES, NOVEMBER 12, 1985

Dr. Gottesman reported on the November 12, 1985, meeting (tab 1260) of the Working Group on Viruses. She said this working group was formed to: (1) address the impact of recent discoveries in virology on the NIH Guidelines; and (2) respond to scientific issues originating in a 1984 memorandum from the Assistant Secretary for Planning and Evaluation, Department of Health and Human Services (DHHS).

Dr. Gottesman said the working group at the November 12, 1985, meeting discussed whether to modify the NIH Guidelines to cover explicitly RNA, whether experiments involving non-infectious viral DNA in tissue culture might be exempted from the NIH Guidelines, and whether Appendix B should be revised to reflect more accurately the situation with retroviruses.

Containment conditions and review procedures for experiments involving viral genes are specified in several sections of the NIH Guidelines: (1) Under Appendix C in certain conditions, viral genes may be propagated and maintained in cells in tissue culture, or may be cloned in E. coli, S. cerevisiae, or B. subtilis host-vector systems. These experiments are exempt. (2) Under Section III-B, viruses classified in Appendix B can be used under specified containment which ranges from BL2 to BL4. The IBC must approve of the experiments before initiation. (3) Under Section III-C, Experiments That Require IBC Notice Simultaneously With Initiation of Experiments, experiments involving viruses are also covered. Section III-C contains a "caution" indicating that special care should be used in evaluating containment levels for experiments involving eukaryotic viral DNA.

Dr. Gottesman said under the current NIH Guidelines most experiments involving retroviruses may be performed under BL2 containment conditions. There is, however, a "caution" in Section III-B-3 which states:

"Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions which permit a productive infection. In such cases, serious consideration should be given to raising the physical containment by at least one level."

The working group felt that the recommendation is appropriate particularly if the vector is non-defective, oncogenic, and has a broad host range.

Dr. Gottesman said a possibility would be to charge this "caution" to a requirement. She said she would ask the members of the Working Group on Viruses to consider developing such a proposal for modification of the NIH Guidelines.

Dr. Cohen suggested the mode of transmission of the virus should also be considered in designating containment levels. Dr. Joklik agreed.

Dr. Gottesman said the working group generally concluded that cloning of an oncogene in bacteria does not present a serious risk.

Dr. Gottesman said the working group also discussed the probability of rescue of a defective retroviral vector through recombination with an endogenous virus. The working group discussion indicated that restraints on retrovirus processing and maturation make it unlikely that an infectious retrovirus carrying a cloned piece of genetic information would be generated. The general rule for retroviruses appears to be if the virus is oncogenic, it is defective; if the virus is nondefective, it is not oncogenic. There are, however, cases in which a virus carries an oncogene and is infectious.

Dr. Gottesman said she had reached the conclusion at the working group meeting that knowledge in retrovirology is accumulating very rapidly although many aspects of retroviral biology are currently unknown. An appropriate working group should continually monitor the situation regarding retroviruses and the NIH Guidelines.

Dr. Friedman, who had participated in the November 12, 1985, meeting, said he felt some concern over the possibility a retroviral vector might recombine with endogenous human retroviral sequences to form a novel virus. He asked whether any full-length endogenous retroviruses have been identified in the human genome.

Drs. Anderson, Miller, and Joklik said they were not aware that any full length retrovirus had been identified in the human genome although attempts have been made to induce such viruses. Knowledge in this area, however, is partial, and one should proceed with surveillance and careful evaluation.

Dr. Anderson said the retroviral vectors currently being considered for use in human gene therapy are based on murine retroviruses. These viruses have very little homology with human sequences, and the probability of recombination with human endogenous sequences is therefore very low. This issue will be carefully evaluated by the Working Group on Human Gene Therapy.

Dr. Miller said human gene therapy vectors will be highly defective, nonhomologous with human endogenous retroviral sequences, and unlikely to be rescued by recombination. These vectors present different issues than oncogenic viruses which are currently being studied in the laboratory and which are not being considered for use in human gene therapy. He pointed out that retroviruses have been studied for over 50 years predating recombinant DNA. He asked if any investigators had acquired disease from such laboratory studies. Dr. Joklik said he knew of no such evidence.

Mr. Mitchell suggested the Working Group on Viruses should continue to meet since knowledge in this field is accumulating very rapidly.

### VIII. REQUEST TO CLARIFY ASSIGNMENT OF CONTAINMENT LEVELS

Mr. Mitchell said Ms. Iclal Hartman, the Chair of the Biohazards Committee of Lexington, Massachusetts, has requested (tab 1258) that RAC clarify its intent when it sets containment at "BL2 containment plus BL3 practices."

Dr. Hartman said these recommendations of "mixed" containment levels pose problems for oversight committees such as the Lexington Biohazards Committee. Many local ordinances mandate compliance with the NIH Guidelines; they also prohibit experiments requiring BL3 or BL4 containment.

Dr. Korwek asked if it would help if a statement were added to the NIH Guidelines indicating that when the NIH Guidelines specify a mix of containment and practices, the containment designation is the primary designation. Dr. Gottesman preferred that RAC offer an interpretation rather than modify the NIH Guidelines. Dr. Korwek agreed and moved that the sense of the RAC is that "where there is a mix of practices and containment, the level of designation refers to containment." Dr. Johnson seconded the motion. Dr. Talbot clarified that this does not change the NIH Guidelines but is merely a RAC recommendation to towns interpreting local ordinances. In cases in which the NIH Guidelines mix practice levels and containment levels, the RAC recommends the town consider the primary designation to be the level of containment.

Mr. Mitchell called the question. By a vote of eighteen in favor, none opposed, and no abstentions, the motion was carried.

### IX. ANNOUNCEMENTS

Mr. Mitchell informed RAC that the Health Research Extension Act of 1985 (tab 1257) mandates establishment of a Biomedical Ethics Board composed of six senators and six representatives. This board will appoint an advisory committee to review ethical problems pertaining to biomedical research including developments "in recombinant DNA technology which have implications for human genetic engineering."

### X. REPORT FROM HUMAN GENE THERAPY WORKING GROUP

Dr. Walters reported that the Working Group on Human Gene Therapy met on December 16, 1985. He said this meeting was primarily an educational session for the working group.

Dr. Walters said Dr. W. French Anderson of the NIH presented a review of the construction of retroviral vectors. Dr. Anderson described the structure of retroviruses and explained how the virus can be modified to create a vector to carry a gene into cells. Modifications which would permit the vector to infect a cell but not replicate and produce infectious particles capable of infecting other cells were also described.

Dr. Walters said Dr. Gottesman then reported to the Working Group on Human Gene Therapy on the November 14, 1985, meeting of the Working Group on Viruses. The Working Group on Viruses had raised no issues related to human gene therapy that were not already addressed in the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols," adopted by the RAC in September 1985.

Dr. Walters said Dr. George Scangos of the Johns Hopkins University then described the current status of transgenic animal studies. The working group learned that the current means of producing transgenic animals are inefficient. Transgenic animal studies are being done: (1) to look at tissue specific development and gene expression; (2) to study the phenotypic effects of foreign gene expression; (3) to study how oncogenes function; (4) to understand how mutations can disrupt gene function; and (5) to create disease models. Dr. Walters said the working group requested this report to attempt to monitor the status of research involving animal germ line modification.

Dr. Walters said Dr. Samuel Ackerman of the Food and Drug Administration (FDA) described the processes for submission to FDA and FDA review of applications for investigational new drugs (IND). Dr. Walters reported the working group learned that: pre-IND discussions between investigators and the FDA are permitted and encouraged by the FDA; and FDA considers the contents and even the existence of an IND application to be confidential information. The working group was reconfirmed in its view that the RAC and its Working Group on Human Gene Therapy will perform a valuable function in their review of human gene therapy protocols which will supplement the FDA review.

Dr. Walters said the working group believes it is important to attempt to formulate a description of the purpose and the contents of the "Points to Consider" in lay language. The working group is in favor of inviting any research group that is intending to submit a protocol to submit preclinical data.

Dr. Walters said proposals to perform human gene therapy would have to be submitted to ORDA about 15 weeks in advance of a RAC meeting. This time-frame will be necessary to permit publication of an announcement of the proposal in the Federal Register and review by the working group.

Mr. Mitchell thanked Dr. Walters for his presentation. He asked when the working group intended to finish their work on a lay summary of the purpose and contents of the "Points to Consider." Dr. Walters said he hoped a draft would be ready by the spring of 1986.

#### XI. SCIENTIFIC SESSION ON HUMAN GENE THERAPY

Mr. Mitchell said the next item on the agenda was a scientific session designed to inform RAC members of the principles, processes, and procedures which might be involved in human gene therapy. [Rapporteur's Note: Attachment II contains a glossary of terms and pertinent illustrations and tables.]

### A. Prospects for Human Gene Therapy

Mr. Mitchell called on Dr. David Martin to begin by discussing prospects for human gene therapy.

Dr. Martin said he would describe the candidate diseases theoretically amenable to gene therapy and address the three major issues associated with human gene therapy: (1) efficacy; (2) safety; and (3) ethics.

Dr. Martin said efficacy in human gene therapy depends on five considerations (1) the appropriate gene must be identified and isolated; (2) the gene must be introduced stably into an expression system; (3) appropriate tissue specific expression must be obtained; (4) an appropriate level of expression must be obtained, i.e., sufficient expression to reverse a phenotype but not so much as to cause detrimental effects; and (5) the disease should have a reversible phenotype. It does not do any good to correct a disease biochemically unless the phenotype will be corrected in a way that justifies the effort and exposure.

Three questions can be posed concerning reversibility of phenotype. The first question is "what is the phenotype?" The second question is "what is the pathogenesis of the disease?" An understanding of the phenotype and the pathogenesis would suggest whether the phenotype is reversible. The third question is "have previous therapies been successful and do these attempts provide information about reversibility of phenotype?"

Dr. Martin said the three primary candidate diseases for human gene therapy are recessive diseases caused by enzyme deficiencies; i.e., the defective gene does not produce a functional enzyme.

[Rapporteur's Note: Higher organisms package their DNA into segments called chromosomes. There are two copies of each of 22 chromosomes (called autosomes) 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." Therefore, in normal human cells, there are 46 chromosomes: two sex chromosomes and two copies of each of the 22 other chromosomes. The egg cells or ova contain 22 chromosomes and an X chromosome. The sperm contain 22 chromosomes and either an X chromosome or a Y chromosome.

The chromosomes carry the genetic information necessary to direct the processes of the cells composing the body. Some of the genes in the approximately 100,000 genes contained in the chromosomes of a human cell may be "faulty" and contribute to or cause disease in the individual possessing the defective gene. A defective gene can be passed to offspring. The diseases caused by faulty genes can be classified by the pattern of inheritance. They can be recessive, dominant, or X-linked.

Recessive diseases occur when the offspring receives two copies of a defective gene, one from each of the parents. Usually, the parents

are unaffected; they both carry one copy of the defective gene but are protected by the presence of one normal copy of the gene. Such individuals are called carriers. Each child of this couple has a 25 percent risk of inheriting a double dose of the defective gene; a 25 percent chance of inheriting two normal copies of the gene; and a 50 percent chance of being a carrier like the parents.

Dominant diseases occur when offspring receive one copy of a defective gene from either parent and having just one copy of the gene leads to expression of the disease.

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; they have no second X chromosome to balance the effects of a defective copy of a gene carried on the X chromosome. Males inherit their X chromosome from their mother and their Y chromosome from their father. If a mother is the carrier of one normal X chromosome and one X chromosome carrying a faulty gene, each male child has a 50 percent risk of inheriting the faulty X chromosome and a 50 percent chance of inheriting the normal X chromosome of their mother. Each female child has a 50 percent risk of inheriting the faulty X chromosome and becoming a carrier like her mother.]

Dr. Martin said dominant diseases, i.e., diseases due to the presence of an abnormal gene dominant to the normal gene, would be far more complex to treat with human gene therapy than recessive diseases. It is hypothesized for recessive diseases that introduction of the normal allele of the defective gene should allow, at least biochemically, for some correction of the disease phenotype. In a dominant disease, for successful treatment by gene therapy expression of the abnormal gene would have to be interrupted. Although some progress is currently being made in learning to integrate genetic material into specific chromosomal sites of mammalian cells, the technology needed to specifically and reproducibly interrupt expression of an abnormal gene is still not available.

Dr. Martin said two of the candidate diseases for human gene therapy, adenosine deaminase (ADA) deficiency and purine nucleoside phosphorylase (PNP) deficiency, are autosomal recessive diseases; i.e., the affected genes are on autosomes. The enzyme purine nucleoside phosphorylase is lacking in PNP deficiency; the enzyme adenosine deaminase is absent in ADA deficiency. In the normal individual, ADA and PNP are present in every tissue of the body. The other candidate disease, the Lesch-Nyhan Syndrome, is caused by a defective gene on the X chromosome. Biochemically, Lesch-Nyhan Syndrome patients are deficient in the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRTase). HGPRTase is an enzyme normally ubiquitous in tissues of the body. It is absent from every tissue in Lesch-Nyhan Syndrome children.

Dr. Martin said these three diseases are the primary candidates for human gene therapy because: (1) the disease presents a unique phenotype recognizable by a mother; and (2) since easy assays exist for these three enzymes, the diseases have been extensively studied and the

genes which express adenosine deaminase, purine nucleoside phosphorylase and HGPRTase have been isolated.

Dr. Martin then described the phenotype of these three diseases. He said the deficiencies of ADA and PNP are present at birth. Some of the children afflicted by ADA deficiency are born immunodeficient. For other ADA-deficient children, the immunodeficiency develops somewhat later in life. The immunodeficiencies associated with PNP deficiency are not usually present at birth; they develop at approximately 12 to 18 months of life. In both ADA and PNP deficiency, the thymus, if present at birth, subsequently involutes, i.e., disappears. Thymus-dependent lymphocytes will also decline concomitant with disappearance of the thymus gland.

The clinical course of both ADA and PNP deficiencies involves recurrent infections, usually of viral origin. Many of these children die of chicken pox. These children are particularly susceptible to viral infections because the T lymphocytes (T cells) which are dependent upon the thymus are responsible for resistance to viral infection. In both ADA and PNP deficiency, T cells are markedly reduced or absent once the disease is full-blown. If ADA afflicted children survive long enough, they will also lose their B cells which are responsible for humoral immunity. A major difference between ADA and PNP deficiency is that PNP deficient children do not have a defect in their humoral immunity; B cell function is normal.

Although the deficient enzymes are absent from every tissue in the body of afflicted individuals, the immune system is the only organ system specifically affected. In PNP deficiency, only a portion of the immune system, the thymic arm, is affected.

Dr. Martin said the Lesch-Nyhan Syndrome presents as a broad spectrum of disease. The clinical phenotype was originally described as a cerebral palsy with choreoathetosis. A choreoathetosis is an incoordinate movement, particularly the coarse movement, of large muscles. Lesch-Nyhan Syndrome patients are mentally retarded although the degree of retardation varies through a spectrum. Most of these patients, but not all, have a very bizarre self-mutilation syndrome, e.g., biting their lips, biting their fingers, scratching their face. These patients produce too much uric acid (hyperuricemia) and develop gout at a very young age, and they usually die of renal failure due to the accumulation of sodium urate and uric acid and concomitant nephritides provoked by kidney stones. Because they are physically disabled, they are prone to developing pneumonia.

Although the major symptoms of the syndrome, choreoathetosis, mental retardation, and self-mutilation, are due to central nervous system involvement, there is no recognized anatomical or biochemical abnormality unique to the central nervous system.

Dr. Martin said prior to six months of age Lesch-Nyhan Syndrome is very difficult to detect clinically although it can be detected biochemically. The clinical disease usually commences at about six months of age and can be recognized by 12 months of age. At three years of age, the disease is clearly evident; and as it progresses, severe secondary contractures of the hamstrings and self-mutilation become evident.

Dr. Martin said this pattern poses a question which must be considered before gene therapy is attempted on children suffering from Lesch-Nyhan Syndrome: is there a point in the development of this phenotype beyond which the disease is no longer reversible?

Dr. Martin said the enzyme deficiencies in these three candidate diseases occur in three consecutive biochemical steps of the same metabolic pathway (Attachment II, Figure 1). Adenosine deaminase catalyzes the deamination of adenosine and deoxyadenosine to make inosine and deoxyinosine. Inosine is a substrate from which purine nucleoside phosphorylase makes hypoxanthine. Hypoxanthine is the substrate for HGPRTase. HGPRTase converts guanine or hypoxanthine to GMP or inosine monophosphate (IMP); IMP is the major precursor for the purine nucleotides ultimately used in the synthesis of DNA.

Dr. Martin said the pathologic effects of an enzyme deficiency can generally be attributed to: (1) insufficient product or secondary product; (2) toxicity from substrate accumulation or from an abnormal metabolite of the accumulated substrate.

Dr. Martin said abnormal metabolites accumulate in ADA and PNP deficiencies. He described the mechanism of toxicity in PNP deficiency. The enzyme purine nucleoside phosphorylase catalyzes the phosphorolysis of four substrates: inosine, deoxyinosine, guanosine, and deoxyguanosine. Patients lacking purine nucleoside phosphorylase accumulate all four of these substrates in their urine and plasma. These substrates circulate and can cross cellular membranes through a facilitated transport system. One of these four substrates, deoxyguanosine (dG), is a quite potent pretoxin. Deoxyguanosine can enter cells and be phosphorylated to the nucleotide, deoxyguanosine triphosphate (dGTP). Phosphorylated nucleotides such as dGTP accumulate in cells since phosphate groups prevent diffusion across membranes. Deoxyguanosine triphosphate inhibits the enzyme, ribonucleotide reductase, which is necessary for forming precursors for DNA synthesis. In the absence of purine nucleoside phosphorylase, DNA synthesis is inhibited and cells do not proliferate. Thymocytes must proliferate in order to differentiate to normally functioning suppressor and helper T cells.

The pathogenesis of ADA deficiency is probably similar. In the absence of adenosine deaminase, adenosine and deoxyadenosine accumulate and can diffuse in and out of cells by a facilitated transport system. Deoxyadenosine is a more potent pretoxin than adenosine; it accumulates as deoxyadenosine triphosphate (dATP), which is also a potent inhibitor

of an enzyme necessary for producing substrates for DNA synthesis. When adenosine deaminase is absent, deoxyadenosine circulates, accumulates in thymocytes as dATP, and prevents DNA synthesis and proliferation. This may not be the whole story of the pathogenesis of ADA deficiency. Some evidence suggests that adenosine triphosphate (ATP) and nicotinamide adenine nucleotide (NAD) levels may be decreased in some cells and variation in these levels may contribute to some of the immune dysfunctions seen in ADA deficiency.

In the ADA deficient patient, ATP accumulates, dGTP accumulation is not observed; in the PNP deficient patient, dGTP accumulates, dATP accumulation is not observed. The abnormal accumulation of these nucleotides can be detected in erythrocytes and lymphocytes. The pretoxins can get in and out of every cell, and they clearly cross the blood-brain barrier.

Dr. Martin then discussed the evidence supporting the hypothesis that a toxin may be produced in the Lesch-Nyhan Syndrome and attempted to identify the pretoxin. Although uric acid is overproduced in Lesch-Nyhan Syndrome patients, the pretoxin is not uric acid, because uric acid levels in Lesch-Nyhan Syndrome children can be reduced biochemically or pharmacologically with no effect on the major signs of the syndrome. Animals treated with uric acid do not display the Lesch-Nyhan Syndrome. In addition, there are other diseases in which urate levels are very high in infancy, and those children do not have neurologic abnormalities.

Dr. Martin then compared the pathogenesis of Lesch-Nyhan Syndrome to the pathogenesis of PNP deficiency in an attempt to identify the pretoxin. Children afflicted with PNP do not usually have neurologic abnormalities while children afflicted with Lesch-Nyhan Syndrome have neurologic abnormalities. In both PNP deficiency and the Lesch-Nyhan Syndrome, purines are massively overproduced. In PNP deficiency, hypoxanthine is not converted to IMP because the body cannot make hypoxanthine. In the Lesch-Nyhan Syndrome, hypoxanthine is not converted to IMP because the enzyme HGPRTase is missing.

Could the pretoxin in Lesch-Nyhan Syndrome be hypoxanthine or some derivative of hypoxanthine? There is one very intriguing series of experiments involving exposure of experimental animals to large doses of hypoxanthine and xanthine derivatives. Rats or rabbits treated with an LD50 of methylated xanthines will self-mutilate, chewing their paws. Methylated xanthines apparently induce a syndrome that in some ways mimics the Lesch-Nyhan Syndrome. The most effective methylated xanthine for inducing a self-mutilation syndrome in these animals is caffeine.

Dr. Martin then discussed the efficacy of previous therapies in reversing the phenotype of the three candidate diseases. Bone marrow transplantation is curative in ADA deficient patients if an HLA matched donor is available as shown by a number of criteria, e.g., T cell and B cell functions are quantitatively and qualitatively normalized. Red

blood cell infusions, i.e., taking blood from normal individuals, irradiating the blood to kill the lymphocytes, and infusing the erythrocytes into ADA-deficient children, are efficacious in about half of the treated children. The patients regain an almost normal number of T cells and T cell functions; and in many cases, transiently regain B cell function. In the presence of normal erythrocytes, the high dATP levels decline and ATP levels increase and stabilize. The immune system reconstitutes but will subsequently decline; dATP will reaccumulate. Reconstitution of the immune system is transient because red cells normally die with time; and as the infused erythrocytes are destroyed, the toxins reaccumulate.

These experiments show it is not necessary to introduce adenosine deaminase into thymocytes. All that is needed to reconstitute the immune system is detoxification of the environment by removal of the pretoxin. If the environment is detoxified, adenosine deaminase deficient thymocytes or precursor thymocytes can repopulate the immune system. A number of children have been carried for several years with such treatment. Eventually, however, the reconstituted immune system develops antibodies to the infused red cells.

Dr. Martin said other therapies have been employed in attempts to treat these children, e.g., parenteral or oral administration of uridine or deoxycytidine. Some indications of possible efficacy have been observed with these therapies, but these observations are not convincing.

Bone marrow transplantation is curative for PNP deficient patients if an HLA matched donor is available. PNP deficient patients have been transfused with erythrocytes with less impressive results than with ADA deficient patients, but such procedures offer clear indications that the immune system of PNP patients can be transiently reconstituted by transfusion with normal red blood cells. Parenteral or oral administration of uridine or deoxycytidine has no effect.

Dr. Martin said a bone marrow transplant has been tried as therapy in HGPRTase deficiency and will be discussed by Dr. Parkman. Exchange blood transfusions have no effect. Adenine was administered several years ago to one of a set of identical twins afflicted with the Lesch-Nyhan Syndrome. There was no observed effect. Various neuroactive pharmaceuticals have been tried. Occasionally there are claims of symptomatic improvement; the evaluation, however, is subjective. Allopurinol, which considerably diminishes hyperuricemia, has no effect on central nervous system (CNS) function in Lesch-Nyhan Syndrome patients. There are variable subjective observations suggesting 5-hydroxytryptophan may be somewhat efficacious in some Lesch-Nyhan Syndrome patients.

Dr. Martin said these studies point out a serious problem in evaluating the effect of a therapy on patients suffering from the Lesch-Nyhan Syndrome; there are currently no good objective criteria to determine whether a therapy mode is efficacious. Such criteria are necessary to evaluate the efficacy of human gene therapy. At the moment, the

most promising means of quantitating an aspect of the Lesch-Nyhan Syndrome employs positron emission tomography (PET) scans to evaluate glucose metabolism in the brain. Glucose metabolism in the brains of Lesch-Nyhan Syndrome patients was compared to glucose metabolism in the brains of normal individuals by Drs. Pallela and Kelley at the University of Michigan. The PET scans show that glucose metabolism in the caudate nucleus in the brains of Lesch-Nyhan Syndrome patients is significantly reduced as compared to activity in the normal brain. If the rate of glucose metabolism within the caudate nucleus normalized against the cortex is plotted versus the age of the individual in years, normal individuals fall within a certain range. The Lesch-Nyhan Syndrome patients fall well below the normal range with the exception of one patient. While this patient has the other symptoms of Lesch-Nyhan Syndrome, he has normal mental function.

The caudate nucleus is involved in gross motor movement, and reduced activity in the caudate nucleus is consistent with the observation of choreoathetosis in children with Lesch-Nyhan Syndrome.

Dr. Martin said the next crucial question is "what enzyme level will be sufficient to reverse or prevent the symptoms of these candidate diseases without causing detrimental effects?"

How much adenosine deaminase would be necessary to ameliorate the symptoms of ADA deficiency? Kung tribesmen of South Africa have a polymorphism in their inbred population. They have about 5 to 10% of normal adenosine deaminase activity in their lymphocytes. These individuals are immunologically normal. In addition, it has been observed that some ADA deficient patients are helped if 5 to 10% of their erythrocytes are replaced by infused normal erythrocytes. Thus, 5 to 10% of the normal expression level appears to be sufficient to ameliorate symptoms in ADA deficient patients. How much is too much adenosine deaminase? There is a disease, a hemolytic anemia, in which some individuals have a 30 to 70 fold increase in ADA activity. Presumably these levels are the upper limit of normal. Therefore, the window of efficacy for ADA is probably somewhere between 5 and 5000% of normal adenosine deaminase activity.

Much less is known about the enzyme levels which might be therapeutic for PNP deficiency. Heterozygote carriers who have 50% of the normal level of purine nucleoside phosphorylase are asymptomatic. There are two brothers in Halifax who have approximately 0.5% of the normal level of PNP. These brothers both have a rather mild immunodeficiency disease. One of them has survived chicken pox. So for PNP, probably somewhere in the vicinity of 5% or more of normal levels of purine nucleoside phosphorylase will be needed to affect symptoms.

What range of enzyme level would be therapeutic for the Lesch-Nyhan Syndrome? Two brothers who have between 5 and 9% of the normal level of HGPRTase have an unusual compulsive aggressive behavior; they both have been incarcerated several times. This observation suggests it is

probably going to take 10% or more of normal levels to ameliorate the behavioral abnormalities. However, some percentage above 1% of normal levels may ameliorate some of the symptoms of Lesch-Nyhan Syndrome.

A critical question is where does that enzyme activity have to be in the Lesch-Nyhan Syndrome patient's body? Does it have to be expressed in a tissue specific pattern in the brain? Would enzyme in the blood be sufficient if circulating levels would destroy the pretoxin?

Dr. Martin said the issue of tissue specific expression appears to be less important in considering gene therapy of ADA and PNP. In ADA and PNP deficiency, it is clearly not necessary for the gene to be specifically expressed in thymus tissue; infusion of normal red cells is an efficacious therapy. It is also known that the pretoxins, deoxyadenosine in ADA deficiency and deoxyguanosine in PNP deficiency, can diffuse in and out of cells.

Dr. Martin said the question of whether tissue specific expression is necessary to affect the CNS aspects of the Lesch-Nyhan Syndrome might be addressed by an HLA histocompatible bone marrow transplant. Could such a transplant prevent occurrence of the disease or the appearance of symptoms if the child were treated early enough? Could treatment reverse some of the signs and symptoms of the disease?

Dr. Martin said since women are "mosaics," studies on female carriers of the Lesch-Nyhan Syndrome might provide answers to some questions. He explained that all women have two X chromosomes. Very early in embryogenesis in females one of the two X chromosomes, either the maternal X chromosome or the paternal X chromosome, is inactivated. Approximately half of a woman's cells will have an active X chromosome from the father, the other cells will have an active X chromosome from the mother. Female carriers of the Lesch-Nyhan Syndrome have one X chromosome carrying a defective HGPRTase gene and another X chromosome that has a normal HGPRTase gene. These women are not, however, mosaic throughout; only the normal X chromosome can be found in the erythrocytes of carriers of Lesch-Nyhan Syndrome. Apparently there is some selective disadvantage to erythrocytes or erythrocyte precursors that do not express normal HGPRTase activity. It is a relative rather than an absolute selective disadvantage; if it were an absolute disadvantage, Lesch-Nyhan syndrome patients would have no red blood cells or they would be severely anemic. Lesch-Nyhan Syndrome patients are not anemic except for some occasional secondary vitamin deficiencies.

Female Lesch-Nyhan Syndrome carriers are neurologically normal; there are no detectable neurologic abnormalities in Lesch-Nyhan carriers even though clinicians can detect mild abnormalities in individuals with partial HGPRTase deficiencies. Are female carriers mosaic in brain tissue or is HGPRTase deficiency selected against in brain tissue? If carriers are not mosaic in their brain, as they are not in their erythrocytes, there would appear to be a distinct selective disadvantage for HGPRTase deficient brain cells during development.

The existence of a selective advantage might effect the outcome of gene therapy. There is one caveat to this hypothesis; cells very closely abutted have "tight junctions" between them and can to some extent exchange nucleotides, specifically IMP, back and forth across their cell borders. It is thus conceivable that in a Lesch-Nyhan Syndrome carrier, cells that have HGPRTase "cross-feed" those that do not express the enzyme and could account for the lack of CNS symptoms in carriers of the Lesch-Nyhan Syndrome.

Dr. Martin said the safety issues associated with human gene therapy can be grouped in essentially four general categories: (1) Is there any potential acute toxicity due to gene therapy? (2) Could replication competent recombinant viruses develop? (3) Could cancer develop as a result of gene therapy? (4) Could a degenerative disease develop as a result of gene therapy? Both cancer and degenerative disease could be caused by insertion of the retroviral vector into the chromosome (insertional mutagenesis).

Dr. Martin described one approach to building safer retroviral vectors; the introduction of a "kamikaze" gene or a "suicide" gene into the vector. The "suicide" gene would be a gene which encodes a unique enzyme that would convert some non-toxic compound to a toxin. Cells harboring the kamikaze vector could be eliminated by feeding the patient a drug toxic only to cells harboring the vector and thus capable of converting the nontoxic drug to a toxic drug.

Although such a "suicide" gene has not been tested directly, Dr. Martin described a related experiment. The enzyme cytosine deaminase converts 5'-fluorocytosine to 5'-fluorouracil. Mammalian cells do not have an enzyme such as cytosine deaminase so they cannot convert 5'-fluorocytosine to 5'-fluorouracil. 5'-fluorouracil, an inhibitor of RNA synthesis, is a widely used chemotherapeutic agent in the treatment of cancer. The Japanese have purified cytosine deaminase, injected it stereotactically into brain tumors of humans, and then fed the patients 5'-fluorocytosine. 5'-fluorouracil was generated in the vicinity of the tumors but not in other parts of body. The brain tumor cells were killed by this therapy. The systemic toxicity associated with 5'-fluorouracil therapy was minimal in these patients and limited to cells in the immediate area into which cytosine deaminase was injected.

Dr. Martin said the deficiencies of ADA and PNP and the Lesch-Nyhan Syndrome are all rare diseases. Yet many investigators have built their careers on investigating rare diseases. Rare diseases can teach an enormous amount about normal physiology and pathology. He quoted William Harvey:

"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows tracings of her work

apart from the beaten path. Nor is there any better way to advance the proper practice of medicine than to give our minds to discovery of the usual law of nature by careful investigation of cases of rare forms of disease."

Dr. Epstein asked Dr. Martin how many diseases he thought might be amenable to human gene therapy. Dr. Martin said currently there are only five or six diseases amenable to human gene therapy. The genetic defect responsible for the phenotype must be known, the phenotype must be reversible and the gene identified and isolated. He thought several other recessive diseases would become candidates for human gene therapy within the next few years. Ultimately a few hundred recessive diseases might be treatable by somatic cell human gene therapy.

Dr. Davis asked why diseases involving expression of abnormal hemoglobin were inferior candidates at this time for human gene therapy. Dr. Martin replied that genes involved in hemoglobin production must be expressed in a finely-tuned, tissue specific manner. The technology is not yet available to achieve such expression.

#### B. Gene Transfer Using Retroviruses

Mr. Mitchell asked Dr. Dusty Miller to describe aspects of retroviral vectors.

Dr. Miller said retroviral vectors are a good method for transferring genes into somatic cells for several reasons: (1) Retroviruses can efficiently transfer genes into large numbers of cells in a population. (2) Stocks of many hundreds of milliliters (mls) containing  $10^6$  to  $10^7$  particles per ml can be generated to produce an enormous number of transfer vehicles. (3) Cells infected by retroviruses are not extensively damaged. (4) The linear organization of the viral genome is preserved upon integration into the host DNA even though retroviruses integrate at random sites. (5) Once integrated, retroviruses become an integral part of the genome and behave like cellular genes. (6) Retroviruses contain strong transcriptional elements, enhancers and promoters, and provide other signals required for transcription so they can provide all the elements necessary to express genes inserted into the retrovirus vector. (7) A variety of sizes of foreign DNA (anything from about 1 kilobase or less to over 7 kilobases) can be packaged in a retroviral vector. A wide variety of spliced versions of gene(s) can be used. (8) Replication defective retroviral vectors can be introduced into cells in the absence of helper virus; in that situation, they will not propagate further. Thus, if cells in culture are exposed to retroviral vectors and subsequently reintroduced into the patient, there is very little potential for spread of the vector to other tissue. (9) Retroviruses can infect a wide variety of cell and tissue types. Retroviral vectors can be constructed to infect mouse, rat, cat, dog, monkey, and human cells; these vectors can be tested in animal models and used directly in humans. (10) High-titer

stable vector-producing cell lines can be constructed, kept in a freezer, and thawed when needed. The cell line used to produce a retroviral vector for a human gene therapy protocol could be certified and would not have to be constructed each time the vector was needed.

Dr. Miller said retroviruses are positive strand RNA viruses, i.e., an RNA genome encodes retroviral protein products. Upon entering the cytoplasm, the single-stranded RNA molecule is reverse transcribed into a DNA form, termed the provirus (Attachment II, Figure 2), which integrates into the genome of the infected cell. Long terminal repeats (LTRs) are present at the junctions between cellular DNA and viral genome. Each LTR contains strong transcriptional elements or regulatory signals necessary for expressing a gene, e.g., "enhancers," sequences that can enhance the transcriptional activity of flanking viral or cellular DNA, promoters, and other signals required for viral transcription. For example, transcriptional elements such as CAT and TATA boxes are in the LTRs; transcription initiation begins in the 5' LTR site; and the 3' LTR contains a polyadenylation site.

The major proteins of the retroviral coding regions are the gag proteins which are involved in virion assembly and structure, pol proteins which are involved in reverse transcription, and env proteins which are involved in virion absorption/penetration and are the primary determinants of retrovirus host range. These proteins are made from RNA transcripts which run the full length of the virus. There is also a shorter-length transcript for the viral env protein.

Dr. Miller then explained how a typical retroviral vector was constructed (Attachment II, Figure 3). He said the gene encoding the enzyme, hypoxanthine guanine phosphoribosyltransferase (HGPRTase), was linked to the retroviral LTRs so that the regions coding for viral protein were either completely removed or truncated. No viral proteins could be expressed by the vector. The vector construct has very little viral information, is replication defective, and must be "rescued" by helper virus or by packaging lines in order to produce virus particles.

Dr. Miller said it would be useful in human gene therapy applications to generate replication-defective particles for use as vectors without generating helper virus. Strategies to produce such vectors in the absence of helper virus rely on "packaging lines." Such a strategy is shown in Attachment II, Figure 4.

Dr. Miller then showed a slide (Attachment II, Figure 4) demonstrating that packaging lines can produce helper-free vector particles. A retroviral vector carrying resistance to methotrexate (DHFR) was introduced into cells of a packaging line. Almost all of the clonal cell lines produce DHFR virus. The lines vary in viral titer produced. Titers of helper virus produced by these packaging lines are at least a million fold less.

Dr. Miller said the assay is important in determining whether packaging lines produce helper-virus free vector. Several types of assays are available. One standard assay measures reverse transcriptase activity in cells infected with virus produced by the packaging cell line. Reverse transcriptase assays are relatively insensitive in the sense that the lower detection limit is probably no fewer than a hundred particles in a ml of supernatant. In a second assay for helper virus, virus to be assayed is used to infect cells harboring a transforming virus, and these cells are mixed with a flat, non-transformed rat cell line. Virus production is observed as the production of "foci" (Attachment II, Figure 5). Nontransformed cells form a flat monolayer and stick tightly to the dish; they do not pile up. In areas where a transforming virus is produced, a localized "focus" is observed as transformed cells round up and come off the dish. Theoretically, any cell in the population carrying a helper virus is detected by such an assay; thus, the sensitivity of this assay is said to be on the order of one particle per ml. A similar assay that avoids the use of transforming viruses tests the ability of the vector to induce virus production. A cell line is exposed to the test virus and virus production from those cells is measured. Dr. Miller said these assays could be used to test bone marrow cells and blood for the presence of helper virus.

Dr. Miller said retroviral vectors can efficiently transfer nonselectable genes and their regulatory elements. This capability is an important consideration in developing human gene therapy vectors. He described the construction of a retroviral vector containing a rat growth hormone (GH) minigene controlled by its own regulatory elements. Regulation of protein expression from an inducible region as well as homogeneous production of the particular protein was sought in cells infected with the vector. If the regulatory elements can be transferred and are active, protein production from the GH gene should be responsive to substances such as dexamethasone or thyroid hormone which are inducers for growth hormone production. The rat growth hormone gene has an internal polyadenylation site and was fused to its own transcription promoter (Attachment II, Figure 6) before insertion into the vector. The human HGPRTase gene was also introduced into the construct in order to permit selection of infected cells. The HGPRTase gene was under transcriptional control of the retroviral LTR.

The GH-HGPRTase vector was used to infect cells lacking the HGPRTase gene. Several independent clones were selected on the basis of HGPRTase activity. All the selected clones secrete growth hormone in the un-induced state and respond to normal inducers for growth hormone production by increasing their growth hormone synthesis. Reasonable levels of expression of growth hormone were observed, i.e., ten to 40 fold lower than cultured rat pituitary cells expressing growth hormone. Cells not infected with the virus produce less than one nanogram (ng)/ml of growth hormone. This experiment shows consistent transfer of the gene, responsiveness of the gene to inducers, and efficient and stable propagation of functional genes and their regulatory sequences in retroviral vectors.

Dr. Miller said several other cell lines were infected with the vector and have been shown to inducibly express the growth hormone gene. In some lines, however, widely variable expression of the growth hormone gene was observed. Some clones express the GH gene at high levels in the absence of induction. Some clones show a 2.4 fold induction. Some clones appear to make very little if any GH even though all clones were selected on the basis of HGPRTase expression. A similar expression pattern may be observed in human gene therapy protocols since the target organ, the hematopoietic system, is composed of diverse cell types. The hierarchy of cells in the hematopoietic/ lymphoid system includes various lymphoid and myeloid elements including T cells, B cells, erythroid cells, granulocytes, macrophages, platelets, etc. A bone marrow transplant represents a more complicated situation than infecting cells in culture.

Dr. Miller then demonstrated that a gene such as the rat GH minigene and its regulatory controls can be transferred by the vector in an unrearranged fashion into cultured cells. Restriction enzymes that either cut outside the vector or in each LTR were used to treat the DNA of infected cells. The restricted DNA was separated on an agarose gel and transferred to a nitrocellulose filter which was then probed for the presence of sequences homologous to the GH gene or the HGPRTase gene. Fragments containing single new integrations could be detected on the gel. The cell lines contained a single GH-HGPRTase provirus of the correct size. These experiments and the inducibility of the GH gene suggest the viral vector can be transferred in an unrearranged fashion.

Dr. Miller then described a protocol for infecting mouse marrow with a retroviral vector. A mouse is first treated with an agent that causes damage to the bone marrow. The damage causes bone marrow cells to replicate and is necessary since retroviruses only infect replicating cells. Five days after injury the marrow of the treated animal is removed and the marrow cells overlaid onto vector-producing packaging cells in culture. After a period of co-cultivation, the marrow cells are harvested, grown for a period of time in the presence of a selective agent, and subsequently assayed for the presence of vector carrying a selectable drug resistance marker. Most of the cells at this point will form drug resistant colonies in vitro and contain the vector DNA.

In principle a human gene therapy protocol is relatively simple. Bone marrow would be removed from the patient, infected with the retroviral vector, and transfused back into the patient who has received whole body irradiation or chemotherapy to ablate the bone marrow and provide a physiological space for the autologous gene inserted bone marrow cells. However, several questions should be addressed before proceeding with human gene therapy. One useful application of retroviruses in basic science has been the demonstration that the pluripotent stem cell will give rise to all of the cells of the mouse hematopoietic/ lymphoid system. The pluripotent stem cell can be detected by the presence of the retroviral vector and its descendents tracked.

However, obtaining consistent expression of the integrated vector in all the different cell types has been a problem. Only a few of the mice actually express the gene carried on the retroviral vector. It is not easy to infect 100% of the mice and to obtain expression in all the cells derived from the pluripotent stem cell.

It is not known whether human pluripotent stem cells would be infected by the retrovirus vector. Although infection of pluripotent stem cells has been demonstrated in mice, it is not clear that pluripotent stem cells from humans or any other large animal can be infected by retroviral vectors. Even if the pluripotent stem cell or some cell derived from it is infected by the vector, the vector and its genes may be inactivated during normal differentiation of the infected cell. It is not known whether inactivation might occur.

Retroviral gene transfer is currently best understood in the mouse system, and there is still a great deal to learn from this system. In dogs, monkeys, or human cells in culture, less is known. For example, for animals other than mice, expression in the various cells of the hematopoietic system has not been demonstrated at all.

Dr. Miller said at this time it is not known if the amphotropic vectors will work in other animals. All current vectors are based on murine leukemia viruses. These vectors work particularly well in mice; however, it is not known whether other animals can be efficiently infected by these vectors. There have been suggestions that primate retroviruses be used for human gene therapy protocols, but gene transfer experiments involving primate retroviruses have not yet been done.

Efficient transfer of vectors into large animals must also be demonstrated. A large animal differs considerably from a mouse. In experiments with mice, one animal is sacrificed; all of its bone marrow is taken, treated, and transplanted into a genetically identical animal. In a larger animal such as a human, marrow must be taken from the patient and returned to the same individual. Quite a large number of cells must be infected and represent a considerable scale-up from the infection protocol used with mice.

Currently, it is not known how long human bone marrow cells can be cultured in vitro and still engraft after transfusion back into the patient. To date the longest period marrow has been cultured outside of a host is on the order of 24 hours.

The persistence of the transferred genes in other animals must also be demonstrated. Persistence of expression has been measured in mice, but currently there are not enough mouse data to justify a human experiment. Experiments in mice suggest there are fairly substantial problems in obtaining reasonable expression levels. Several groups have reported low expression of vectors carrying the adenosine deaminase or purine nucleoside phosphorylase genes in mouse marrow cells.

Problems could also arise with the technique used to generate efficient infection of the cells. In experiments that work well with mice, bone marrow cells are co-cultivated with virus-producing cells. The protocol would be cleaner if bone marrow cells could be cultured with a supernatant containing the vector since it may not be easy to remove co-cultivated cell line elements from the treated bone marrow cells. The possibility of contamination with these elements is an added variable which should be evaluated.

Finally, it is not known whether in human gene therapy protocols the bone marrow must be ablated by radiation or chemotherapy. It may not be necessary to ablate the marrow in human gene therapy protocols since the patient will be transfused with autologous tissue. Whether selectable markers and in vivo selection pressure could be employed is another question which should be addressed.

One negative aspect of retroviral vectors is the potential for mutation. Mutations can be categorized as: (1) Those that will result in death of the cell. These mutations are not a concern because those cells will disappear, and some cells may be lost without affecting the function of the organ. (2) Those that activate an oncogene (insertional activation) or deactivate a dominant gene which restricts cell growth (insertional deactivation). Insertional activation or deactivation could result in transformation. Animal studies to date have not yet clearly addressed the issue of insertional deactivation. Whether insertional activation will occur with a fairly high frequency should be tested. Available mouse data do not suggest a high frequency, but much larger numbers of vectors and cells will be used in human therapy than in mouse protocols.

The problem of insertional activation could be avoided if site specific recombination, i.e., the ability to alter specifically a gene, were possible; however, site specific recombination with high efficiency is technically not feasible at the present time.

With all of these unanswered questions, Dr. Miller thought submission of a protocol to the NIH for review would not likely occur within the next six months.

Dr. Miller said the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" are well designed. Several committees of experts will probably be required to review technical issues. Technical expertise is needed for all the different issues in human gene therapy, e.g., vector construction, ethics, viral recombination, efficacy, and safety.

Dr. Gottesman asked what percentage of retroviral vectors are rearranged internally upon integration into a chromosomal site. Dr. Miller said it is difficult to determine a percentage based on the types of assays he had described. However, using a vector which encodes HGPRTase and factor IX, a blood clotting factor, 40 HGPRTase positive cell clones had been screened to determine whether factor IX was also made from the viral vector. Thirty-nine clones produced factor IX; one clone did not.

C. Role of Allogeneic Bone Marrow Transplantation in Correction of Genetic Diseases

Mr. Mitchell asked Dr. Robertson Parkman to describe allogeneic bone marrow transplantation and the potential role of autologous bone marrow transplantation in the treatment of genetic diseases.

Dr. Parkman said allogeneic bone marrow transplantations predict which genetic diseases will benefit from autologous gene inserted transplants (human gene therapy). Currently bone marrow transplantation can be used to treat three classes of disease: (1) cancer that does not respond to standard therapy; (2) diseases in which stem cells are absent; and (3) diseases in which defective stem cells are present. In this third category, the types of abnormalities which can be successfully treated by allogeneic bone marrow transplantation can be due either to defects in structural genes, to defects in the regulation of gene expression, or to defects in differentiation. Human gene therapy or autologous gene-inserted transplants would appear to be applicable to genetic diseases in this third category.

Genetic diseases can be categorized by response to allogeneic bone marrow transplantation (Attachment II, Figure 7). The first category is comprised of diseases in which the expression of the genetic defect is restricted to bone marrow derived cells; most of these diseases involve defects in red blood cells, the immune system, or granulocytes. The disease can be corrected by ablating the abnormal bone marrow and replacing the abnormal stem cells with normal stem cells.

Diseases in the second category are characterized by a generalized defect expressed throughout the body. The abnormal products accumulate in either bone marrow derived or non-bone marrow derived cells, and small amounts of exogenous enzymes administered by infusion or organ graft appear to reduce or reverse the accumulation of the abnormal substrate. Storage diseases are in this category.

The third category consists of generalized or restricted genetic defects in which an abnormal product accumulates in non-bone marrow derived cells and these accumulations are not reduced by the administration of exogenous enzymes. Diseases such as cystic fibrosis, hemophilia, and phenylketonuria are in this category. This third category of diseases would not be curable by bone marrow transplantation.

Dr. Parkman said patients suffering from genetic diseases in which the missing gene product is normally expressed in hematopoietic and lymphoid derived cells are most likely to benefit from transplantation of histocompatible normal bone marrow. Ablation of the abnormal stem cells and replacement with normal histocompatible stem cells results in permanent and complete correction of genetic diseases in this category. No cases of successfully transplanted patients drifting back to their disease have been observed.

In those categories of disease amenable to bone marrow transplantation, one of the first issues to be considered is the relationship of heterozygote enzyme levels to clinical phenotype. Following successful bone marrow transplantation from a normal donor, approximately 10% of the bone marrow cells will be of donor origin. Assuming that the amount of enzyme per cell is the same in all cells of the body and that each homozygous cell has 100% of the normal enzyme level, after successful transplantation the patient at best would have 10% of normal total body enzyme activity. Therefore, if levels greater than 10% of normal levels are needed to affect phenotype, transplantation therapy will not ameliorate clinical disease since the transplanted patient will at best produce 10% of normal total body levels.

Dr. Parkman said the relative amount of enzyme in circulating cells as compared to the target tissue must also be considered. If circulating cells have one "measure" of enzyme and the target tissue normally expresses 100 "measures," it is unlikely that an adequate level of enzyme could be transported into the target tissue to produce a clinically significant effect. An active as opposed to a passive transport mechanism appears to be an important factor in moving circulating enzyme to intercellular sites.

Results with allogeneic bone marrow transplants will probably be superior to results with autologous gene inserted bone marrow transplants. If clinical stabilization or improvement of the patient cannot be produced by a procedure in which every transplanted cell is homozygously normal, given all the limitations it is not probable that autologous inserted bone marrow transplantation would have a potential therapeutic role in treating that disease.

Dr. Parkman said the bone marrow in post-natal life in humans appears to be composed of two populations, lymphoid stem cells and hematopoietic stem cells (Attachment II, Figure 8); it is not clear whether the pluripotent stem cell from which both groups are derived is present in humans in post-natal life. A bone marrow transplant in humans is, therefore, a transplant of two separate stem cell populations. One or the other or both populations can engraft, and this has a great deal of clinical significance.

[Rapporteur's Note: It is hypothesized that pluripotent stem cells are the undifferentiated progenitor cells of hematopoietic stem cells and lymphoid stem cells. Cells such as erythrocytes, macrophages, and granulocytes differentiate from hematopoietic stem cells. Lymphocytes and plasma cells are derived from the lymphoid stem cells.

The progenitors of macrophages, the promonocytes, originate in the bone marrow; these cells are released into the circulation as monocytes. Monocytes comprise approximately 3 to 8 percent of the circulating leukocyte population. An even greater number of leukocytes, estimated to be approximately three times the circulating population, exists in a marginal pool consisting of monocytes adhering to epithelial surfaces.

Upon entering tissue, monocytes develop into macrophages. Macrophages kill bacteria, fungi, and tumor cells. They also function in the induction of the immune response by processing and presenting immunogenic material to lymphocytes.

Two separate populations of lymphocytes differentiate into the lymphoid cells involved in humoral (B cells) and cellular (T cells) immunity. These populations become restricted to lymphoid differentiation prior to migration to the thymus where T cells are generated or to the fetal liver and adult bone marrow where B cell development occurs. This phase, termed primary differentiation, reflects a genetically programmed sequence of differentiation.

B cells represent approximately 15 to 20 percent of the normal peripheral blood lymphocytes, 50 percent of the splenic lymphocytes, and 75 percent of the lymphocytes in the bone marrow. Their chief role is the production of antibodies. The first recognizable step in development of B cells is the emergence of lymphoid cells which contain small amounts of intracytoplasmic IgM but lack the surface immunoglobulin receptors characteristic of mature B lymphocytes. These pre-B cells are first generated in fetal liver and are found almost exclusively in the bone marrow of adults. Pre-B cells proliferate rapidly and spawn immature B lymphocytes which express surface IgM receptors. Lymphocytes committed to synthesis of IgD, IgG, IgA, and IgE are all derived from IgM-bearing precursors through a genetic switch mechanism.

Stem cells destined to become T cells proliferate in the bone marrow and migrate to the thymus where they acquire the characteristics of T cells. Once the T cells have left the thymus, this gland may continue to exert an effect on them by secretion of thymic hormones (thymosin or thymopoetin). Approximately 70 to 80 percent of normal peripheral blood lymphocytes and 90 percent of lymphocytes in thoracic duct fluid are T cells. They circulate primarily as long-lived small lymphocytes. T cells are involved as helper or suppressor cells in modulating the immune response and regulating B lymphocyte differentiation. T cells that have become sensitized to foreign substances are the principal effectors of cell mediated immunity. Secondary differentiation follows stimulation of specific clones of lymphocytes by antigen.

Because of the pivotal role played by T lymphocytes and the complex interactions involved in immune responses, immune deficiencies primarily involving T cells are usually also associated with abnormal B-cell function. Conversely, immunodeficiencies manifested primarily by inability to produce antibodies may be caused by T-cell defects not associated with abnormal cell-mediated immunity.]

Dr. Parkman said in most transplants the marrow is taken by needle from the posterior iliac crest of the donor. The bone marrow is then filtered through a series of stainless steel screens and transfused intravenously into the recipient. Stem cells have the capacity to "home" to the bone marrow; presumably their surface carbohydrates direct their migration.

A "physiological space" has to be available if transplanted stem cells are to engraft in the recipient. It is not known what "space" means biochemically, but functionally a hematopoietic engraftment will not occur without prior treatment to eliminate resident hematopoietic stem cells. When the recipient's hematopoietic stem cells occupy the physiological space, transplanted hematopoietic stem cells do not engraft. They wither and die. For this reason, drugs or whole body irradiation are administered prior to transplantation to ablate the recipient's bone marrow.

An immune deficiency syndrome, adenosine deaminase (ADA) deficiency, was the first genetic disease to be successfully treated by bone marrow transplantation. T lymphocytes are markedly reduced or absent in children afflicted with ADA deficiency. In 1968, a child who did not receive any treatment to prepare a "physiological space" was transplanted with allogeneic donor marrow. The lymphoid stem cells of the donor engrafted; donor hematopoietic cells did not engraft.

Dr. Parkman said bone marrow transplantation therapy was next attempted on a patient afflicted with the Wiskott-Aldrich Syndrome. Wiskott-Aldrich Syndrome is an X-linked recessive disorder with immunological components; the symptoms are eczema, thrombocytopenia with platelets of reduced size and function, reduced T cell function, decreased capacity to produce antibodies to carbohydrate antigens, T lymphocytes deficient in a structural glycoprotein GPL-115, and platelets with an abnormal glycoprotein. Most Wiskott-Aldrich Syndrome patients do not survive childhood; they die of complications of bleeding, infection, or malignancy.

Dr. Parkman said the effects of the Wiskott-Aldrich Syndrome are due to dysfunction of both lymphoid and hematopoietic stem cells. The Wiskott-Aldrich Syndrome was the first genetic disease with a hematopoietic component for which correction by bone marrow transplantation was achieved. The first transplant patient was prepared by administration of cyclophosphamide, a drug used to eliminate lymphoid stem cells, and ARA-C, a drug it was hoped would eliminate hematopoietic stem cells. These drugs were administered in order to create "physiological space."

Following the drug preparative regime the patient's white count disappeared and he was transplanted with bone marrow from a female sibling with blood type "A." After transplantation the patient's white and red cell count rose. Normally a month after transplantation the patient begins to make platelets. However, a month after transplantation this patient was entirely dependent on transfusions for platelets. Upon examination, the patient's blood group was shown to be "O," his own.

The Y chromosome was visible in the granulocytes; the granulocytes were male, his own. The patient's own bone marrow cells rather than the donor's were the source of hematopoiesis. For several months after the

transplant the T cells were female but they subsequently reverted to male. In summary, a partial lymphoid graft occurred, no hematopoietic graft occurred, and nine months after transplantation, the patient was back to where he was before the transplant. He never showed any improvement in thrombocytopenia.

The patient was then given anti-thymocyte serum and procarbazine as immunosuppressive drugs and total body irradiation to eliminate hematopoietic stem cells. He was then transplanted a second time with donor marrow from the female sibling. Approximately a month after the transplant, his platelet count began to rise and eventually stabilized at a normal level. His red blood cells were blood group "A." His granulocytes no longer displayed a Y chromosome. All lymphoid and hematopoietic cells were female. Nine years after transplantation the patient is doing fine.

Ninety percent of the people afflicted with Wiskott-Aldrich Syndrome can be cured of their disease if a histocompatible bone marrow donor can be found. Although the nature of this disease has not yet been elucidated, the basic defect in the Wiskott-Aldrich Syndrome is thought to be the absence on lymphocyte surface membranes of a glycoprotein with a molecular weight of 115,000 daltons. An autologous gene inserted bone marrow transplant could be attempted on Wiskott-Aldrich Syndrome patients if the gene coding for this glycoprotein could be isolated.

Dr. Parkman said the original Wiskott-Aldrich Syndrome patient received total body irradiation to ablate the hematopoietic stem cells. However, some ethical issues are associated with irradiating children. For this reason, a preparative regime involving the drugs cyclophosphamide and busulfan was developed. Cyclophosphamide eliminates lymphoid stem cells; busulfan eliminates hematopoietic stem cells. Currently, most patients are prepared for bone marrow transplantation with drug preparative regimes.

Dr. Parkman said bone marrow transplantation was then used to treat Kostmann's Syndrome, infantile agranulocytosis. Children with this syndrome are born without granulocytes. They make normal red blood cells and platelets; however, the granulocytes progress 80% of the way through differentiation and then stop. The genetic defect that blocks differentiation of the granulocytes at this stage is unknown. These patients contract fulminant infections and usually die of these infections.

The patient was prepared with the drugs ATS and procarbazine to eliminate lymphoid stem cells and TPI to eliminate hematopoietic stem cells. After transplantation, granulocytes of donor origin were observed to have engrafted. Eight years after transplantation the patient continues to have functioning donor granulocytes.

Kostmann's Syndrome is one of a variety of primary defects in granulocyte function which produce patients who suffer from recurrent bacterial infections. These defects can be successfully treated by allogeneic bone marrow transplantation and could be treated by autologous gene-inserted bone marrow transplants if the primary defects were known and the genes isolated.

Dr. Parkman said therapy with bone marrow transplantation was next attempted on diseases of the red cells; thalassemia is the primary model. Thalassemia is a disease of abnormal gene regulation; hemoglobin chains are made in an inappropriate fashion.

If a histocompatible donor is not available, current therapy for thalassemia involves red cell transfusions every 3 to 4 weeks and a drug, Desferal (desferoxamine mesylate). The drug is administered to remove excess iron which builds up from the chronic transfusions and which can damage the heart. Both transfusions and Desferal present risks. The quality of life is improved by this therapy, but people afflicted with this disease still often die in their early 20's.

If the patients are young children who have not been heavily transfused and do not have secondary problems, an 85 to 90% success rate can be achieved with allogeneic bone marrow transplantation.

The challenge for autologous inserted gene transplantation therapy in treating diseases such as thalassemia is to develop the capability to insert normal regulatory genes to appropriately control alpha and beta hemoglobin chain expression.

Dr. Parkman said there are several types of red cell differentiation diseases including pure red cell aplasia. Three children with pure red cell aplasia were recently transplanted with donor bone marrow. All three children now make red cells and are no longer doomed to receiving red cell transfusions every few weeks and Desferal. Differentiation defect diseases of the red cells could potentially be treated by gene inserted autologous bone marrow transplants if the primary defects were understood and the genes isolated.

In addition to red cells, platelets, and T and B lymphocytes, mononuclear cells are derived from the bone marrow. Mononuclear phagocytic cells and Kupffer cells, the fixed phagocytic cells of the liver, are bone marrow derived. Fixed phagocytic cells in the bone marrow are bone marrow derived. The lung macrophages are derived from bone marrow; and it appears that the microglial cells, the phagocytic scavenger cells in the brain, are also bone marrow derived. Following successful transplantation, these bone marrow derived cells would also be of donor origin. In generalized enzyme deficiency diseases such as lysosomal storage diseases, these donor cells can function as "cellular liposomes" which carry enzymes throughout the body.

The lysosomal storage diseases include most of the lipid storage disorders, the mucopolysaccharidoses, the mucopolipidoses, and glycoprotein storage diseases. These enzyme deficiencies have an autosomal recessive basis with one or two exceptions. The accumulated material often causes visceromegaly or macrocephaly, and secondary atrophy can occur, particularly in brain or muscle. Gaucher's Disease is an example of a lysosomal storage disease.

The basic defect in Gaucher's Disease is the lack of the enzyme glucocerebrosidase which is involved in the hydrolysis of glucocerebroside, a component of red cell membranes. Glucocerebroside is normally phagocytized and broken down by macrophages. In Gaucher's Disease, the macrophages phagocytize the membranes but because they lack glucocerebrosidase they are unable to break them down. The macrophages become filled with red cell membranes. These bloated macrophages crowd out the normal bone marrow, fill up the lungs, and create much clinical symptomatology.

Dr. Parkman described transplantation therapy of a patient with Gaucher's Disease. The patient was prepared with the cyclophosphamide/busulfan drug combination and transplanted with donor marrow. Prior to transplantation, the patient had low levels of glucocerebrosidase. After transplantation, the circulating cells had high levels of this enzyme. The research team expected to observe a normal hematopoiesis in the bone marrow at the same time circulating enzymatically normal cells were present, i.e., two months after transplantation. However, two months after transplantation the patient's bone marrow was still filled with abnormal cells. It was not until some six months after transplantation that normal bone marrow function appeared and the bloated macrophages began to disappear. Although the half life of circulating cells can be measured in hours or days, the half lives of tissue macrophages are much longer; six months may be required before they begin to die off.

This observation is particularly important in considering bone marrow transplantation as a therapy for diseases affecting the central nervous system (CNS). If transplant therapy for Gaucher's Disease is a reasonable model, it will be at least six months before any microglial cells of donor origin would be observed in the CNS, and any beneficial effect from bone marrow transplantation would be observed. This is clinically important if the course is one of rapid deterioration. Transplantation can only stabilize a condition. Once a neuron is dead, it is dead.

Dr. Parkman said the primary question for a variety of metabolic diseases is how well, if at all, does enzyme in the circulation enter the CNS. Allogeneic bone marrow transplants stabilize the CNS component of mucopolysaccharidoses because the circulating enzyme can affect non-CNS substrate accumulations; in part because an active transport mechanism exists. The second question is if the enzyme can enter the CNS, will it be able to reverse CNS accumulation of the abnormal product(s)? He hypothesized that improvement in CNS function may be induced even if

circulating enzyme does not cross the blood-brain barrier if microglial cells are of bone marrow origin. Bone marrow derived cells in the brain could act as trojan horses to introduce the needed enzyme into the CNS. At this time, however, it is not known whether microglial cells are indeed bone marrow derived and how rapidly they turn over.

Dr. Parkman said the next disease his group attempted to treat through bone marrow transplantation was the Lesch-Nyhan Syndrome. What symptoms of the Lesch-Nyhan Syndrome could be ameliorated by a bone marrow transplant assuming that 10% of the bone marrow cells would be of donor origin following transplantation? Since people who have 30% of normal HGPRTase activity have problems with uric acid excretion, transplantation would not be expected to affect this aspect of the phenotype. Likewise, people with 10% of normal HGPRTase activity have problems with athetoid movements. People who have very low levels of HGPRTase, i.e., between 1 and 2%, may have gout, hyperuricemia, and athetoid movement, but do not self-mutilate. If, following transplantation, the patient could produce 1 or 2% of normal HGPRTase levels, the self-mutilating behavior might be corrected.

The self-mutilation behavior is the aspect of the Lesch-Nyhan Syndrome most destructive to patients and families. Other aspects of this syndrome such as kidney stones and athetoid movements are clinically and societally more acceptable. It is the compulsive aggressive behavior that keeps these people wheelchair bound, tied down, and unable to go out in public. It is the compulsive aggressive behavior that, in a sense, renders these patients social pariahs. The most significant clinical improvement then would consist of arresting the self-mutilation behavior.

Two assumptions were made in transplanting a Lesch-Nyhan Syndrome child: (1) since there are no observed morphological or anatomical changes in the brain, this disease does not produce any permanent change; and (2) circulating enzyme could detoxify the toxic metabolite affecting CNS function. After preparation with the busulfan/cyclophosphamide drug combination and transplantation, normal levels of HGPRTase were observed in the patient's red cells, white cells, and all lymphocytes. This observation indicates engraftment of both hematopoietic and lymphoid elements. Unfortunately, this patient has displayed no clinical improvement in terms of the compulsive aggressive behavior. Dr. Parkman concluded that either: (1) there is permanent damage to the brain which cannot be detected morphologically; (2) the behavior is learned and after a certain number of years cannot be modified; or (3) the circulating enzyme is not able to detoxify the central nervous system.

Dr. Parkman said his group hopes to transplant an infant afflicted with HGPRTase deficiency since an infant is less likely to have anatomical damage to the brain. If a child under the age of a year successfully transplanted still develops clinical disease, this disease probably is not amenable to reconstitution by either allogeneic or autologous bone marrow transplantation therapy.

The phenotype of a generalized enzymatic defect can be corrected if the critical enzyme can gain access to accumulations of the substrate (Attachment II, Figure 9). If the enzyme is not able to get to the site of accumulation, there will be no phenotypic correction of the disease. Clinicians in Cincinnati recently transplanted a child with Pompe's Disease, a glycogen storage disease. Complete peripheral correction of the deficiency was obtained with bone marrow transplantation; however, glycogen levels in the muscle and liver before and after transplantation were the same. Even though the transplanted marrow cells successfully engrafted and expressed the critical enzyme alpha-glucosidase, the enzyme was not getting from the circulation to the site of substrate accumulation in the muscles.

One recent result suggests CNS deterioration can in some cases be stabilized by allogeneic bone marrow transplantation. Donor derived lymphocytes have been detected in the spinal fluid of a child with metachromatic leukodystrophy successfully transplanted with allogeneic bone marrow. Approximately six months after this child was transplanted, the CNS decay stabilized.

Dr. Parkman then discussed how autologous inserted gene bone marrow transplants would be useful. Only 30% of the individuals who could be cured by a bone marrow transplant have a histocompatible donor, i.e., 70% of the people who have a treatable disease do not have an appropriate donor. This group would benefit from autologous inserted gene transplantation. Second, graft versus host disease and its related complications have a 10 to 20% one year mortality rate. If inserted gene transplants have a reduced morbidity/mortality because the transplanted tissue is autologous, the risk/benefit ratio would be shifted toward benefit. Third, the patient currently must be prepared for allogeneic bone marrow transplantation by complete lymphoid and hematopoietic ablation. With autologous inserted gene transplants, it may be possible to achieve engraftment after selective or sub-total lymphoid or hematopoietic ablation thereby decreasing the toxicity associated with preparation for a transplant.

Dr. Parkman summarized (Attachment II, Figure 9) by indicating that the diseases amenable to bone marrow transplantation are generalized diseases in which the symptomology is restricted to lymphoid and hematopoietically derived cells such as adenosine deaminase (ADA) deficiency, nucleoside phosphorylase (PNP) deficiency, and Gaucher's Disease. These diseases are very good candidates for autologous gene inserted transplantation.

There are other disorders restricted to lymphoid and hematopoietic stem cells that are correctable by bone marrow transplantation. However, the primary defects in most of these diseases have not yet been elucidated. The basic physiology of the disease must be known before these diseases could be treated by autologous gene inserted bone marrow transplantation.

Finally, there is a category of diseases which is not amenable to bone marrow transplantation therapy unless a method is developed to obtain expression of gene products not normally produced by bone marrow cells.

Dr. Cohen asked Dr. Parkman to compare the oncogenic potential of preparative irradiation or drug ablation with the oncogenic potential of insertional activation by the retroviral vectors to be used in human gene therapy.

Dr. Parkman said exposure to 1000 rads of radiation increases the likelihood of developing cancer by 10% over the level inherent in the individual's genetic make-up. The preparative drugs, cyclophosphamide and busulfan, produce sterility and are mutagens which increase the likelihood of developing cancer. However, the likelihood of a 10 year old child surviving aplastic anemia without a bone marrow transplant is 25%. A successfully transplanted child may develop a secondary cancer from the preparative regime 40 years after transplantation; however, the child would have lived many additional years. The consent form states the potential side effects of the therapy.

Dr. Parkman said a family with a member afflicted with a genetic disease treatable by bone marrow transplantation must choose: There is a 20% possibility the child will die from the transplant when he/she would not have died in the short term from the underlying disease. On the other hand, if the transplant is successful, the child will probably lead a full life with normal capacity. Generally if one child in the family has already died of the disease, the family will opt for the attempt to cure the second child rather than having not only to live through the slow decay of that child but also the memories of the decline and death of the first child.

Dr. Walters asked whether a trial and error phase existed in the early stages of bone marrow transplantation. Dr. Parkman said there were 14 survivors among the first 110 leukemia patients treated with bone marrow transplantation therapy. However, those patients received the best therapy available at the time; the fact it did not work for most of them is not the issue. Dr. Parkman said he never performs a transplant as an experiment, rather he does transplantations as the best therapy available at the time to give the patient the best long-term quality of life.

Mr. Mitchell asked Dr. Parkman whether he thought bone marrow transplantation would be effective in older members of the population. Dr. Parkman replied that transplantation therapy of leukemia and aplastic anemia is performed on adults 40 to 50 years of age. However, with age the body is less able to withstand the insult of the preparatory ablation. In addition, the severity of graft versus host disease increases with age. An autologous gene inserted transplant might be able to circumvent graft versus host disease and the amount of drugs necessary to prepare for an autologous transplant may be less.

Mr. Mitchell thanked Drs. Parkman, Martin, and Miller for their presentations.

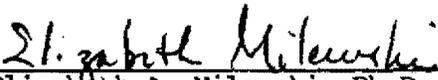
XII. FUTURE MEETING DATES

Mr. Mitchell said the RAC is scheduled to meet on May 12 and September 29, 1986.

XIII. ADJOURNMENT

Mr. Mitchell adjourned the meeting at 4:25 p.m. on January 27, 1986.

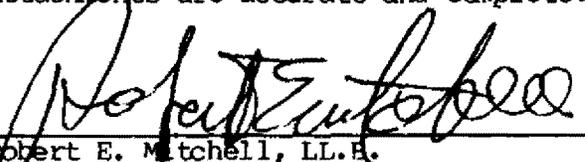
Respectively submitted,

  
Elizabeth A. Milewski, Ph.D.  
Rapporteur

  
William J. Gartland, Jr., Ph.D.  
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

9/29/86  
Date

  
Robert E. Mitchell, LL.B.  
Chair  
Recombinant DNA Advisory Committee

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Glossary of Terms

ablate: to remove.

allogeneic: sufficiently unlike genetically to interact antigenically.

amphotropic: capable of infecting cells from a broad range of different animal species.

anatomy: a branch of morphology that deals with structure.

antigen: protein or carbohydrate substance that when introduced into the body stimulates the production of antibody.

atrophy: decrease in size or wasting away of a body part or tissue.

autologous: derived from the same individual.

chorea: a disorder characterized by irregular, spasmodic, involuntary movements of the limbs or facial muscles.

choreoathetosis: Abnormal body movements of combined choreic and athetoid movements.

clone: a group of genetically identical cells or organisms produced asexually from a common ancestor.

complementary DNA (cDNA): DNA synthesized from a messenger RNA template rather than the usual DNA template.

cortex: the outer portion of an organ such as the brain.

cytoplasm: the protoplasm of a cell, external to the cell's nuclear membrane.

differentiation: the process by which descendants of a common parental cell achieve and maintain specialization of structure and function.

DNA probe: a sequence of DNA that is used to detect the presence of a particular nucleotide sequence.

eczema: generic term for acute or chronic inflammatory conditions of the skin.

erythrocyte: a mature red blood cell.

exogenous: originating from or due to external causes.

expression: the process by which the blueprint contained in the DNA is converted into the structures and biochemical patterns present and operating in a cell.

genome: the total of the genetic information contained in the chromosomes of an organism.

helper virus: a virus which provides components needed for replication and/or packaging of a defective virus.

hematopoiesis: the formation of blood or blood cells in the body.

heterozygote: an animal or plant in which the two genes of at least one genetic locus are different alleles.

histocompatibility: a state of mutual tolerance that allows some tissues to be grafted effectively to others.

homozygosis: gametes identical for one or more pairs of genes.

humoral: relating to the extracellular fluids of the body.

inducer: a substance that is capable of activating a structural gene directly or by combining with and inactivating a genetic repressor.

leukocyte: any of the white or colorless nucleated cells that occur in blood.

lymphocytes: a white blood cell formed in lymphoid tissue throughout the body; e.g., lymph nodes, spleen, tonsils, Peyer's patches and bone marrow.

lymphoid: of, relating to, or constituting the tissue characteristic of the lymph nodes.

macrocephaly: having an exceptionally large head or cranium.

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 protein molecules from the chromosome in the nucleus to the synthetic apparatus in the cytoplasm.

milliliter (ml): a unit of capacity equivalent to one thousandth of a liter, and equivalent to 0.033815 fluid ounce.

morphology: a branch of biology that deals with the form and structure of animals and plants.

mosaic: the juxtaposition in an organism of genetically different tissues.

mutagen: a substance that tends to increase the frequency of mutation.

nephritis: inflammation of the kidneys.

nonselectable gene: a gene to which a means of applying selective pressure does not exist.

phagocyte: a cell (as a leukocyte) that characteristically engulfs foreign material and consumes debris and foreign bodies.

phenotype: a category or group to which an individual may be assigned on the basis of one or more characteristics observable clinically or by laboratory means that reflect genetic variation or gene-environment interaction.

plasma cell: a mononuclear slightly ameboid wandering cell that is usually found in association with low-grade chronic inflammations or with various allergic processes.

platelet: a blood particulate, irregularly shaped containing granules in the central part and peripherally, clear cytoplasm, but no definite nucleus.

pluripotent stem cell: a cell capable of differentiating into all of the cell types present in a given tissue, e.g., hematopoietic tissue.

polymorphism: occurring in several forms.

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

provirus: the integrated DNA form of an RNA virus.

regulatory sequence: a DNA sequence involved in regulating the expression of a gene.

rescue: the process by which a helper virus provides components necessary to the replication and/or packaging of a replication defective virus.

restriction nuclease: an enzyme that has the ability to recognize a specific nucleotide sequence in a nucleic acid and cleave the nucleic acid at that point.

retrovirus packaging cells: cells which provide factors necessary for replication of retroviral vectors.

retrovirus vector: retrovirus designed to transmit specific genes.

reverse transcription: the synthesis of DNA from RNA, the reverse of processing genetic information from DNA to RNA.

selection: a natural or artificial process or pressure that results in the survival and propagation of organisms but not of others with the result that certain traits are perpetuated, e.g., the addition of an antibiotic to a culture to select those organisms which are resistant to the antibiotic.

splicing: the process by which noncoding material in mRNA is removed prior to use of the mRNA in translation.

stem cell: an unspecialized and usually embryonic cell ancestral to one or more specialized cells.

thrombocytopenia: decrease in the number of blood platelets.

thymocyte: a cell that develops in the thymus.

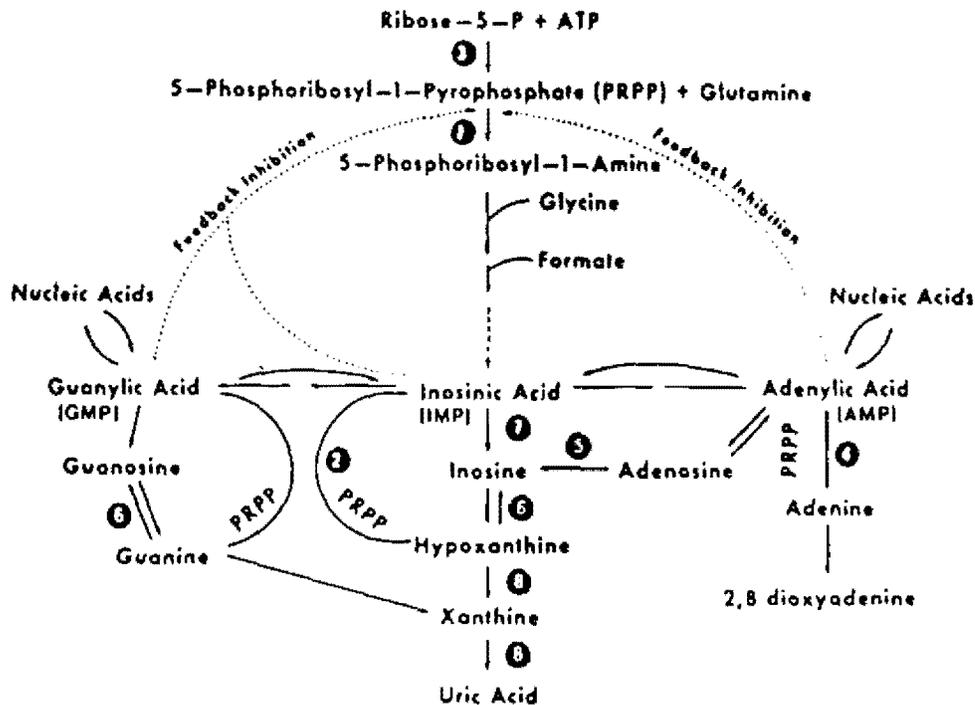
titer: the quantity of a substance in solution as determined by titration, e.g., the number of virus particles per ml.

transfect: the transfer of genetic determinants from one organism or cell to another by means of uptake of the genetic material through the membrane.

transcription: the process by which a complementary messenger RNA molecule is formed from a single strand 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.

visceromegaly: enlargement of internal organs.

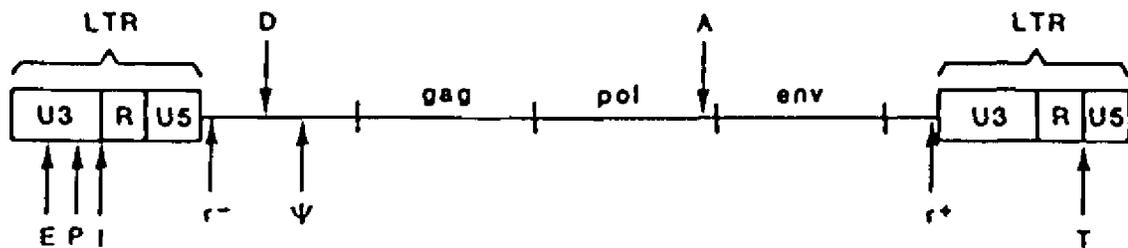
Figure 1

OUTLINE OF PURINE METABOLISM

- (1) amidophosphoribosyltransferase
- (2) hypoxanthine-guanine phosphoribosyltransferase
- (3) PRPP synthetase
- (4) adenine phosphoribosyltransferase
- (5) adenosine deaminase
- (6) purine nucleoside phosphorylase
- (7) 5'-nucleotidase
- (8) xanthine oxidase

Figure 2.

Simplified Structure of Retroviral Proviral DNA.



E: enhancer

P: promoter

I: initiation (Cap) site for viral RNA synthesis

r<sup>-</sup>: replication initiation site for minus DNA strand

D: donor splice site

ψ: packaging sequence

A: the major acceptor splice site

r<sup>+</sup>: replication initiation site for plus DNA site

T: terminal [poly (A) addition] site for viral RNA synthesis

LTR: long terminal repeat

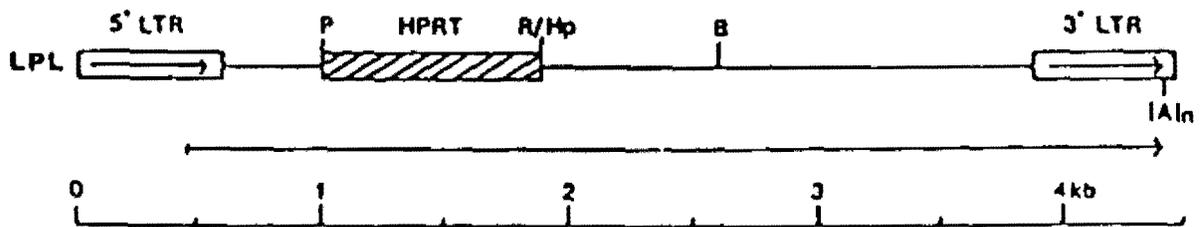
gag: DNA encoding viral core antigens

pol: DNA encoding RNA dependent DNA polymerase (reverse transcriptase)

env: DNA encoding envelope proteins

Figure 3.

HGPRTase - Retroviral Vector Construction



LTR: long terminal repeats

HPRT: HGPRTase gene

Figure 4.

VIRUS PRODUCTION FROM PACKAGING CELLS CONTAINING DHFR-VIRUS

<u>CLONE #</u>	<u>DHFR-VIRUS TITER</u>	<u>HELPER-VIRUS TITER</u>
1	$6 \times 10^5$	<1
2	$6 \times 10^5$	<1
3	$1 \times 10^7$	<1
4	$4 \times 10^6$	<1
6	$3 \times 10^5$	<1
7	$<1 \times 10^3$	<1
8	$5 \times 10^6$	<1
9	$1 \times 10^5$	<1

Figure 5.

S<sup>+</sup>L<sup>-</sup> ASSAY FOR HELPER VIRUS

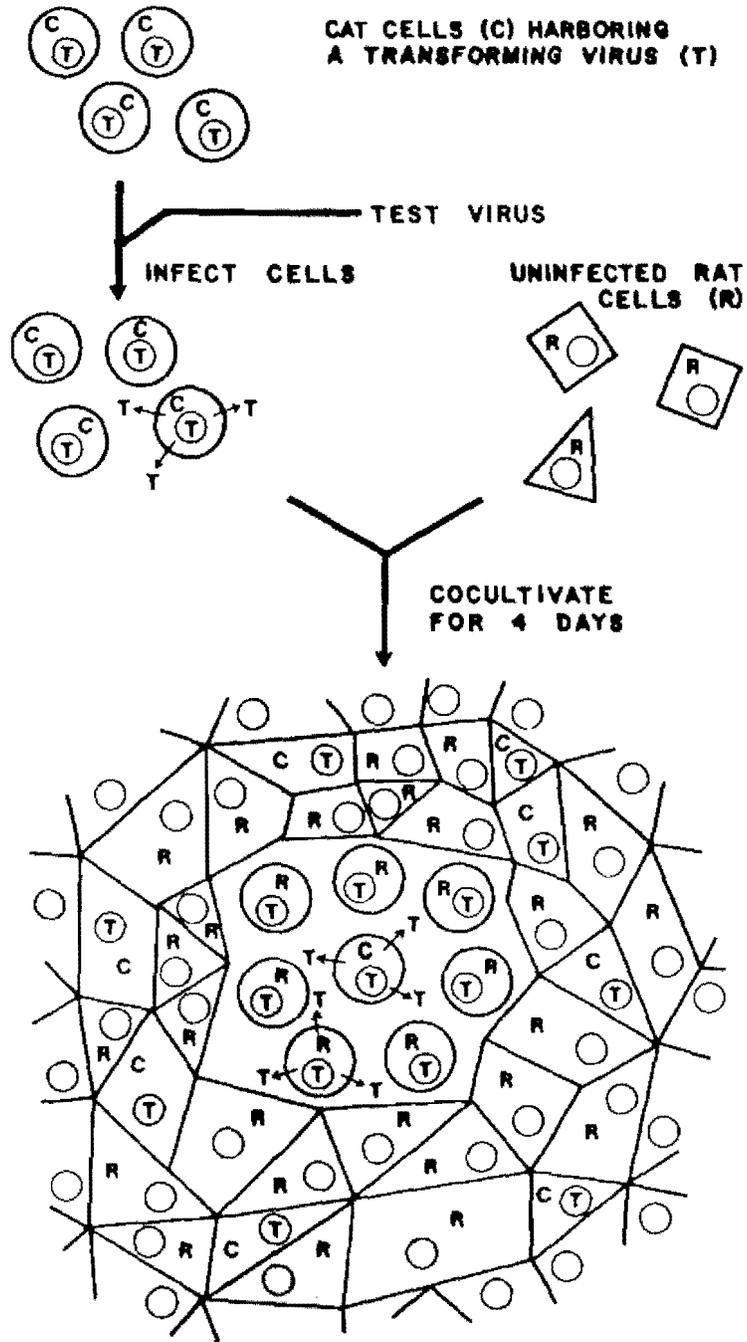
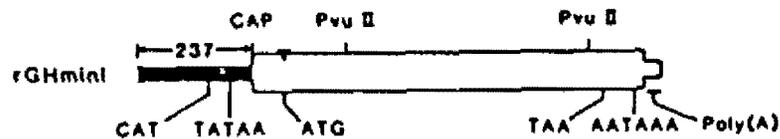
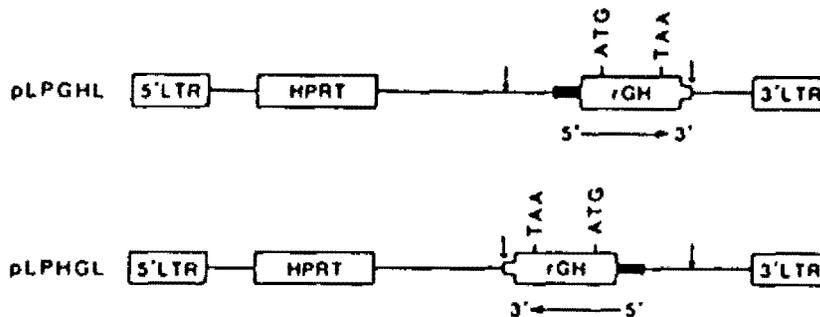


Figure 6.

The GH-HGPRTase Vector Construct



Structure of the rat GH minigene. The GH promoter region (black) was fused to the GH full-length cDNA.



Structure of the viral vectors containing the GH minigene. The structures of viral vectors containing the GH minigene in the same (pLPGHL) or opposite (pLPHGL) orientation relative to viral transcription are shown.

Figure 7.

GENETIC DISORDERS:  
POSSIBLE ROLE OF BONE MARROW  
TRANSPLANTATION AS THERAPY

1	2	3
Expression of genetic defect or abnormal gene product restricted to bone marrow derived cells	Generalized genetic defect; abnormal products accumulate in bone marrow or non-bone marrow derived cells; low levels of circulating enzyme reverse accumulations	Generalized or restricted genetic defect; abnormal products accumulated in non-bone marrow derived cells and are <u>not</u> reversed by circulating enzyme.
Correctable by BMT	May be Correctable by BMT	Not Correctable by BMT
Severe combined immune deficiency Wiskott-Aldrich Syndrome Infantile agranulocytosis Chronic granulomatous disease Thalassemia Sickle cell disease Enzyme-deficiency hemolytic anemias Osteopetrosis Agammaglobulinemia C <sub>2</sub> and C <sub>3</sub> deficiency	Gaucher's disease Fabry's disease Hurler's disease Hunter's disease Cystinosis	Krabbe's disease Metachromatic leukodystrophy GM <sub>1</sub> gangliosidosis Tay-Sachs disease Abetalipoproteinemia Wilson's disease Cystinuria Cystic fibrosis Hereditary angioedema Hemophilia Phenylketonuria Hyperbilirubinemia

Figure 8.

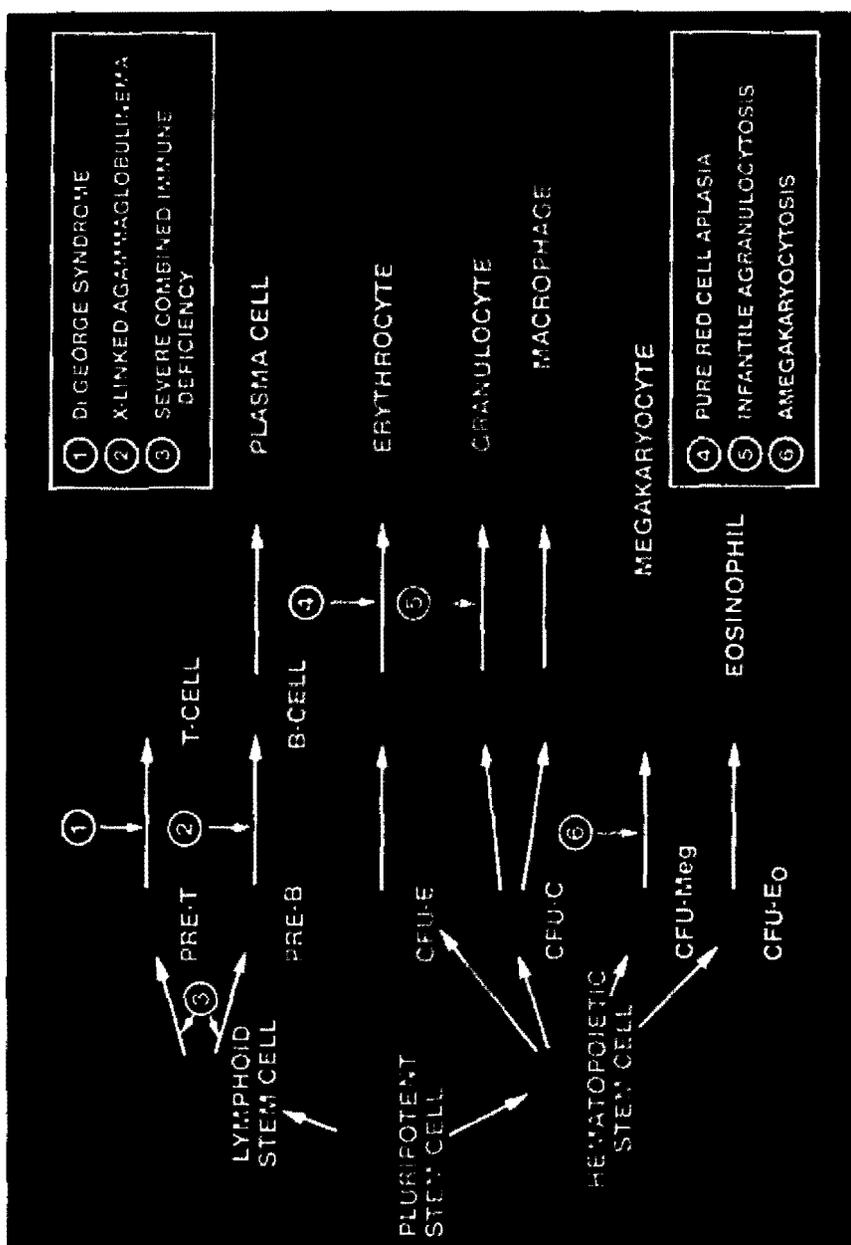


Figure 9.

GENERALIZED ENZYMATIC DEFECTS CAN BE CORRECTED IF LYMPHOID/HEMATOPOIETIC DERIVED ENZYME CAN ACCESS TO INTRA-CELLULAR ACCUMULATION OF SUBSTRATE. THE RELATIVE SUCCESS OF THE EXOGENOUS ENZYME WILL BE DETERMINED BY:

- A) THE RELATIVE AMOUNTS OF ENZYME IN BONE MARROW DERIVED CELLS AS COMPARED TO THE AFFECTED TISSUE
- B) IF ACTIVE TRANSPORT MECHANISMS EXIST FOR THE INTERNALIZATION OF CIRCULATING ENZYME
- C) IF CIRCULATING ENZYME PHYSICALLY GAINS ACCESS TO THE AFFECTED TISSUE PARTICULARLY THE CENTRAL NERVOUS SYSTEM

MUCOPOLYSACCHARIDOSIS HAVE BENEFITED FROM ALLOGENEIC BONE MARROW TRANSPLANTATION BECAUSE THE CIRCULATING BONE MARROW DERIVED ENZYME REACHES THE NON-CNS SUBSTRATE ACCUMULATIONS. THE SUCCESS OF BONE MARROW TRANSPLANTATION IN STABILIZATION OF THE CNS COMPONENT OF THE DISEASES IS STILL UNCERTAIN.

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Becky Lawson

RECOMBINANT DNA ADVISORY COMMITTEE

NATIONAL INSTITUTES OF HEALTH  
BUILDING 31, CONFERENCE ROOM 10  
BETHESDA, MARYLAND

JANUARY 27, 1986

MAILING I



National Institutes of Health  
Bethesda, Maryland 20205  
Building : 31  
Room : 3B10  
(301) 496- 6051

December 23, 1985

MEMORANDUM

To: Members  
Recombinant DNA Advisory Committee

From: Executive Secretary

Subject: January 27, 1986, Meeting - Mailing I

The next meeting of the committee will be on January 27, 1986, at the National Institutes of Health, Building 31C, Conference Room 10, 9000 Rockville Pike, Bethesda, Maryland 20892. The meeting will begin at 9 a.m. This will be a one day meeting.

Room reservations have been made for the evening of January 26, 1986, at the Marriott Hotel in Bethesda (301-897-9400) for those of you who will need accommodations. If you wish to change or cancel these reservations, please contact Ms. Becky Lawson in my office at 301-496-6051. For arrival after 6 p.m., a deposit in the amount of one night's stay is required by either a check in the amount of \$58 or a major credit card authorization. The hotel will not hold the room past 6 p.m. without a deposit.

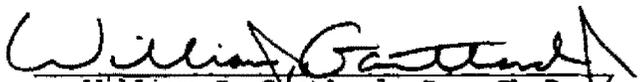
Drs. David Martin, Dusty Miller, and Robertson Parkman will be attending the meeting to participate in a scientific session on human gene therapy. Drs. David Friedman and Mark Saginor will be attending as consultants.

A preliminary list of primary reviewers is included in this mailing.

Enclosed for your consideration are the following documents:

- Dates of future meetings.....1247
- Establishment of Biotechnology Science  
Coordinating Committee.....1248, 1249
- Proposed major actions as submitted to  
Federal Register.....1250
- Background documents on proposed major actions.....1251-1254
- Minutes of September 20, 1985, meeting of Subworking  
Group on Human Gene Therapy.....1255

Please bring all these materials with you to the meeting.

  
William J. Gartland, Jr., Ph.D.

Enclosures

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PRIMARY REVIEWERS

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Dr. Clowes.....1250/I, 1250/II, 1252, 1254  
Dr. Cohen.....1250/II, 1254  
Dr. Davis.....1250/II, 1254  
Dr. Friedman.....1250/I, 1250/II, 1250/III, 1251, 1252, 1254  
Dr. Gottesman.....1250/I, 1250/III, 1251, 1252  
Dr. Joklik.....1250/III, 1251  
Dr. McGonigle.....1250/IV, 1253  
Dr. Mills.....1250/IV, 1253  
Dr. Rapp.....1250/I, 1250/III, 1251, 1252  
Dr. Saginor.....1250/IV, 1253