Ethical Implications of Cancer

No matter where you are or what you do today you will probably come in contact with a carcinogen. A CARCINOGEN is a substance which is thought to cause cancer by producing mutations in the chromosomes of an organism. The list of known carcinogens includes alcohol, asbestos, tobacco, and many other common chemicals. In addition, recent evidence has suggested that we all harbor proto-oncogenes within our cells. PROTO-ONCOGENES are normal cellular genes which lead to cancer when activated by changes in the cell's chromosomes.

It is estimated that 73 million Americans now living will develop cancer sometime during their lives. That means that three out of four families will be affected directly by cancer. Currently, 29.2 million or 40% of those who develop cancer will survive for five years after their cancers are diagnosed. In the United States a death from cancer occurs about every 67 seconds.

With these dismal statistics one can see why millions of dollars are devoted each year to the diagnosis and treatment of cancer. Only within the last ten years, however, has one major goal of cancer research -- how to prevent cancer from occurring -- gained much attention and support.

The first step towards preventing cancer has been to identify those substances which may promote cancer. Two methods are currently used to detect possible carcinogens. In the Ames test, the suspected carcinogen is applied to a culture of Salmonella bacteria that have a mutation which prevents them from producing the amino acid histidine. If the substance applied to the bacteria is mutagenic, some of the
mutations preventing the production of histidine will be reversed. The number of reverse mutations which occur is an indication of the strength of the carcinogen. In the Sister Chromatid Exchange (SCE) test, potential carcinogens are applied to cells growing in culture. The chromosomes of the cells are stained in a way such that the breaking and rearranging of chromatids may be detected easily. Substances which cause a large number of chromatid exchanges are considered carcinogenic. The SCE test can be used to determine a specific individual's sensitivity to a particular substance as well as the sensitivity of the general population.

A second method used to help prevent cancer has been to identify those practices which may reduce the risk of cancer. Eating a balanced diet may be one way to avoid cancer. High fiber foods lower the risk of colon cancer. Cruciferous vegetables (e.g., broccoli, cabbage, and brussel sprouts) contain dithiolthiones which increase the production of glutathione which helps detoxify chemicals in the body. Food rich in vitamins A and C (e.g., carrots, apricots, peaches, and oranges) seem to reduce the risk of many cancers. Evidence also suggests that the risk of esophageal cancer may be minimized by limiting the amount of fat, alcohol and nitrate-preserved meat in the diet. Reducing the amount of tobacco smoked or quitting altogether will lessen the risk of cancer significantly. Avoiding excessive exposure to radiation, including sunlight, has also been shown to reduce the risk of cancer.

Unfortunately, many known carcinogens and many practices which promote cancer have come to be major parts of our lifestyle. Every year we manufacture several million tons of useful, but potentially cancer-causing material. Formaldehyde, a chemical linked to many cancers, is found in everything from plywood to toothpaste. Often a
A substance is released into the air, water, or soil for 10 to 20 years before the effects of the substance are seen. Halocarbons and dioxin are two well-known substances whose affects on the world have just been discovered after years of use. Halocarbons, used as propellants in spray cans, rose into the upper atmosphere and destroyed ozone which shields the earth from ultraviolet radiation. The loss of some of the ozone layer resulted in an increase in the number of skin cancers. Dioxin, a herbicide, was used widely until it was discovered that it increased the occurrence of cancer and other health problems. Today, such places as Time Beach, Missouri and Love Canal, New York, are largely abandoned due to dioxin contamination.

As a society and as individuals we are faced with a very difficult (and ethical) decision concerning how we use the knowledge science has provided us. Consider, for example, the following questions:

1. What do you think is an acceptable level of risk for exposure to carcinogens?
2. What substances in your life (e.g., cigarettes, saccharin, caffeine) would you be willing to give up to reduce your risk of cancer?
3. Do you think that there is a difference between voluntary (e.g., smoking and poor eating habits) and involuntary risks (e.g., herbicides and flurocarbons)?
4. Who do you think ought to determine what substances ought to be regulated or banned?

References Cited

BIO 199  
PERSONAL RISK ASSESSMENT  

I would be willing to give up or reduce mine or others use of the following products and/or practices in order to reduce the risk of cancer to myself and others. (Mark an "X" on the appropriate line to indicate your stance.)

<table>
<thead>
<tr>
<th>Product/Practice</th>
<th>YES</th>
<th>MAYBE</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>sunbathing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>smoking</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>artificial sweetners</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microwave ovens (microwave radiation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>herbicides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pesticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>birth control pills (estrogen)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soda, coffee, tea (caffeine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food colorings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>satellite television (microwave radiation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>multiple sex partners (cancer-causing viruses)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;junk&quot; food (high fat content)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>photocopiers, typewriter ribbons (carbon black)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry cleaning (ethylene dichloride)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food preservatives (nitrates and nitrites)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>climate-controlled buildings (radon and high levels of pollutants)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plastic products (vinyl chloride)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stressful occupations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fertilizers</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
INTRODUCTION TO LEADING DISCUSSIONS*

A task-orientated discussion is one that is directed toward the solution of a particular problem and provides direction and a sense of accomplishment for the participants. Leading a discussion requires very different skills than participating in a discussion. The leader's input is not needed for determining the solution to the problem; rather, it is needed to keep the discussion task-oriented and thus, progressing toward a solution. "The leader is a person seen by the group members he is working with as helping them fulfill their needs."[6] The leader need not do all of the leading; some of the functions may be provided by the group members. According to Miles [6], the functions that must be present are the following: (1) initiation, (2) regulation, (3) information, (4) support, and (5) evaluation.

Certain roles may develop among the participants that will reduce the group's efficiency. The following are some of the disruptive roles that may appear [2]:

(1) Blocker - totally negative; prevents progress
(2) Aggressor - insults and criticizes others
(3) Anecdator - tells irrelevant stories that waste time
(4) Dominator - seeks to monopolize group interaction
(5) Recognition seeker - seeks attention and sympathy
(6) Confessor - seeks counseling for personal problems
(7) Special interest pleader - seeks recognition for a non-relevant cause
(8) Playboy - distracts group with antics, jokes, and comments

All of these roles should be minimized. The aggressor especially should not be tolerated because, for a discussion to be successful, the participants must feel comfortable and non-threatened. Some individuals will be more domineering than others, which is fine, unless they prevent others from entering the discussion. To stop the dominating person, after the dominator makes a point, the leader may quickly say, "Thank you, who can add to what has been said?" The anecdator or anyone who gets off the subject will prevent the group from progressing toward a solution to the problem. To counter this person, the leader may: (1) restate the goal, or (2) ask if what is being said applies to the solution of the problem.

Here are some additional hints:

(1) Sometimes ideas must be rejected for better ideas. Note, the idea, not the person who suggested it, is rejected.
(2) Feelings ought to enter into the discussion and be acknowledged, because even the best solution is unworkable if it is offensive.
(3) Leaders should never answer their own questions, because this will surely stifle discussion.
(4) An extended period of silence may seem uncomfortable, but it will give the participants time to think. Eventually someone will answer the question to ease the tension.

Bio 199

DISCUSSION GROUP ATTENDANCE SHEET

Discussion Group Leader: ________________________________

Discussion Topic: ______________________________________

Date: ________________ Location: _________________________

Discussion Group Leader:

Please record the names of all BIO 199 students in attendance at the discussion group meeting. Turn in this attendance sheet by the Friday following your discussion group meeting. Thank you!

1. ______________________________________________________
2. ______________________________________________________
3. ______________________________________________________
4. ______________________________________________________
5. ______________________________________________________
6. ______________________________________________________
7. ______________________________________________________
8. ______________________________________________________
9. ______________________________________________________
10. _____________________________________________________
FOLLOW-UP QUESTIONS FOR DISCUSSION LEADERS

1. What do you think is an acceptable level of risk for exposure to carcinogens?

A. Some people suggest that society must balance the potential risk of cancer against the potential benefits of the substance.
   1. What type of benefits do you think may be worth the risk of cancer?
   2. Do you think that the person who accepts the benefits ought to experience the risks?
   3. Do you think that others who do not receive the benefits still bear the risk of cancer in the use of some products? Is this fair, right or justifiable?
   4. Do you think greater risks ought to be accepted for greater good?
      For example, ought a fertilizer which increases crop yield by 20% be used even though it has been shown to be highly carcinogenic?

B. Other individuals suggest that no risk is acceptable and that all carcinogens ought to be banned regardless of cost or inconvenience.
   1. Do you think cost ought to be considered in the decision to ban a substance? Is that putting a price, even indirectly, on human life?
   2. Do you think the availability of a satisfactory substitute for a product or practice ought to be considered in the decision to ban a substance?
   3. Do you think that there is any sacrifice too great for good health?

2. What substances in your life (e.g., cigarettes, saccharin, caffeine) would you be willing to give up to reduce your risk of cancer?

Table 1. Estimate of the proportions of cancer deaths that will be found to be attributable to various factors.

<table>
<thead>
<tr>
<th>Percentage of all U.S. or U.K. cancer deaths that might be avoidable</th>
<th>Range of acceptable estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>tobacco</td>
<td>25-40</td>
</tr>
<tr>
<td>alcohol</td>
<td>2-4</td>
</tr>
<tr>
<td>diet</td>
<td>10-70</td>
</tr>
<tr>
<td>food additives</td>
<td>-5*-2</td>
</tr>
<tr>
<td>sexual behavior</td>
<td>1</td>
</tr>
<tr>
<td>yet-to-be-discovered hormonal analogues</td>
<td>0-12</td>
</tr>
<tr>
<td>of reproductive factors</td>
<td></td>
</tr>
<tr>
<td>occupation</td>
<td>2-8</td>
</tr>
<tr>
<td>pollution</td>
<td>1-5</td>
</tr>
<tr>
<td>industrial products</td>
<td></td>
</tr>
<tr>
<td>medicines and medical procedures</td>
<td>0.5-3</td>
</tr>
<tr>
<td>geophysical factors (mostly natural background radiation and sunlight)</td>
<td>2-4</td>
</tr>
<tr>
<td>infective processes</td>
<td>1-?</td>
</tr>
<tr>
<td>unknown</td>
<td>?</td>
</tr>
</tbody>
</table>

*The net effects of food additives may be protective, e.g., against stomach cancer.*
3. Do you think that there is a difference between voluntary (e.g., smoking and poor eating habits) and involuntary risks (e.g., herbicides and fluorocarbons)?

   A. Do you think that products which promote cancer and are voluntarily consumed ought to be regulated or banned? For example, ought companies be allowed to forbid their employees to smoke at work? At home?
   B. Do you think that products which promote cancer and are involuntarily consumed ought to be regulated or banned?
   C. Do you think banning a product which promotes cancer and is voluntarily consumed is an infringement upon the individual's right to self-determination? Does it infringe upon the individual's right to privacy?
   D. What do you think ought to be done when a person voluntarily using a carcinogenic substance begins to infringe upon the right of another individual not to be exposed to that substance (i.e., second-hand smoke)?

4. Who do you think ought to determine what substances ought to regulated or banned?

   A. Who do you think ought to decide what products are banned? The government? Scientists? The public?
   B. How do you think regulations controlling carcinogenic substances ought to be enforced?
   C. Do you think today's society has an obligation to protect future generations from an increased risk of cancer?
   D. What criteria do you think ought to be used to determine a significant level of risk of cancer? The Ames test? The SCE test? Animal studies?
BIO 199
CARCINOGENS, CANCER AND ONCOGENES EVALUATION

Either check the appropriate response, or if you prefer, reply in the space provided.
DO NOT SIGN YOUR NAME.

1. Do you feel that the discussion moved satisfactorily towards a solution to the problem?
   YES _____ NO _____ A solution was reached, but not through a logical process. ____

2. Did the discussion cause you to look at the problem from a viewpoint other than your own?
   YES _____ NO _____ Undecided ____

3. Were you permitted to express your views without hostility or ridicule from others?
   YES _____ NO _____ Undecided ____

4. Did the reading material provide you with enough background information to discuss intelligently the use of potential carcinogens and the effect that they may have on the human body?
   YES _____ NO _____ Undecided ____

5. Do you think that there is an acceptable level of risk for exposure to carcinogens?
   YES _____ NO _____ Undecided ____

6. Do you think that there is a difference between voluntary (e.g., smoking and poor eating habits) and involuntary risks (e.g., herbicides and fluorocarbons)?
   YES _____ NO _____ Undecided ____

7. Do you feel that the discussion was a profitable use of your time?
   YES _____ NO _____ Undecided ____

8. Did the discussion help you form an opinion on the issue?
   YES _____ NO _____ Undecided ____

9. Name of your discussion leader: ______________________________

10. Please list any suggestions you may have to improve the discussion or the reading material.
THE MOLECULAR BASIS OF CANCER QUIZ

1. Activated oncogenes and DNA damage have been found in
   a. all of the cells of all of the cancers studied.
   b. none of the cells of any of the cancers studied.
   c. some of the cells of all of the carriers studied.
   d. some of the cells of only some of the cancers studied.

2. Enhancers increase the rate of
   a. transfection of nearby genes.
   b. translocation of distant genes.
   c. translation of distant genes.
   d. transcription of nearby genes.

3. The inactivation of both alleles of a gene to promote cancer is associated with
   a. proto-oncogenes.
   b. oncogenes.
   c. pseudo-oncogenes.
   d. anti-oncogenes.

4. Chronic myelocytic leukemia (CML) may result from a chromosomal translocation which alters the
   a. regulatory mechanism of the gene product.
   b. the amount of gene product produced.
   c. the structure of the gene product.
   d. the time at which the gene product is produced.

5. Proto-oncogenes often affected by mutations promoting cancer, are genes for
   a. structural components of bone cells.
   b. oxygen-carrying molecules of the blood.
   c. growth factors and their receptors.
   d. pigment in the skin cells.

6. Before both retroviruses and DNA viruses can have any cancer-inducing effect on a cell, they must
   a. insert their genetic material into the host cell’s DNA.
   b. insert their proteins into the host cell’s DNA.
   c. produce numerous viral proteins using the host cell’s ribosomes.
   d. produce numerous progeny identical to themselves.

7. According to the somatic mutation hypothesis, all of the following may explain how oncogenic changes occur EXCEPT
   a. viruses.
   b. present medical practices.
   c. chemicals and radiation.
   d. spontaneous mutations.
8. Insertional mutagenesis involves
   a. placing one of the host’s genes under the control of a viral promoter.
   b. destabilizing the virus’ chromosomes and thereby promoting chromosome breakage.
   c. placing one of the virus’ genes under the control of the host’s promoter.
   d. separating a viral gene and its promoter.

9. The chromosomal translocation thought to be responsible for Burkitt’s lymphoma
   a. places a proto-oncogene under the influence of another gene’s enhancer.
   b. places an abnormal form of a viral gene under the influence of a host gene enhancer.
   c. attaches a normal gene to an abnormal gene to produce an abnormal protein.
   d. attaches a viral gene to an abnormal gene to produce an abnormal protein.

10. Chromosomal translocations may cause the activation of proto-oncogenes by changing the
    a. number of genes involved in the production of the protein.
    b. physical setting of the gene.
    c. way the gene is replicated.
    d. function of the cell in which the translocation occurs.

11. In cases where amplification does not appear until after diagnosis of cancer, amplification appears to be strongly related to
    a. tumor regression.
    b. tumor progression.
    c. patient recovery.
    d. patient death.

12. Oncogenes carried by retroviruses are thought to be
    a. unique viral genes which developed by mutation during evolution.
    b. unique viral proteins which have been highly conserved during evolution.
    c. altered pseudo-oncogenes acquired from some previous infection of the host cell’s genome.
    d. altered proto-oncogenes acquired from some previous infection of the host cell’s genome.

13. Interest in proto-oncogenes, oncogenes and antioncogenes is increasing because
    a. they are easily discovered.
    b. they are present in all cells of all animals.
    c. they may provide useful information for the future prevention, diagnosis, and treatment of cancer.
    d. they are presently known to be responsible for all forms of cancer which afflict man.
14. Genetically inherited abnormalities may predispose a cell to cancer by
   a. increasing the cell size.
   b. lessening the number of steps necessary to produce cancer.
   c. eliminating the cell from the body.
   d. increasing the length of time needed for each mutation leading to cancer to occur.

15. Retroviruses containing oncogenes infect normal cells and produce a malignant phenotype in a process known as
   a. viral mutagenesis.
   b. viral translation.
   c. viral transduction.
   d. viral insertion.

16. Individuals who inherit the deletion of one Rb gene (for retinoblastoma) are likely to develop tumors in both eyes because the production of cancer may require only
   a. a chromosome translocation.
   b. the activation of a proto-oncogene.
   c. the inactivation of the second Rb gene.
   d. the infection of the cell by a virus.

17. An increased risk of chromosomal translocation in cells infected with the EB virus may result from
   a. the large number of viral genes which are inserted into the host’s genome.
   b. the longer period of growth and division of the infected cell.
   c. environmental cofactors such as chronic malaria infections.
   d. all of the above.

18. Mutations in proto-oncogenes may cause the production of proteins which are
   I. slow, but functional.
   II. deformed and semi-functional
   III. normal, but nonfunctional.
   IV. misdirected, but functional.
   a. I and II.
   b. II, III, and IV.
   c. I, II, and IV.
   d. III.

19. Amplification is thought to cause cancer by the increased production of proteins which
   a. inhibit the effects of growth inhibiting factors.
   b. cause the cells to destroy themselves.
   c. enhance the cell’s ability to resist drugs.
   d. enhance the cell’s ability to survive radiation treatments.

20. The activation of a proto-oncogene to a state which may promote cancer is thought to be
   a. a single, irreversible step.
   b. a single, reversible step.
   c. a multistep process.
   d. a completely unknown mechanism.
GENE THERAPY QUIZ

1. The goal of gene therapy is "to cure" a genetic disease

   A. temporarily.
   B. by administering hormones produced in bacteria by recombinant DNA technology.
   C. involving either large segments of chromosomes, polygenic inheritance, or environmentally-induced disease.
   D. permanently.

2. The DNA of cells is "corrected" for use in gene therapy by

   A. deleting a defective or nonfunctional gene from the organism's cells.
   B. supplying to the cells hormones produced in bacteria by recombinant DNA technology.
   C. inserting the DNA of a normal gene into the cells of an organism with a defective or nonfunctional gene.
   D. removing the cells containing the defective or nonfunctional gene from the organism.

3. Somatic cell gene therapy involves changes in

   A. sperm or egg cells which can be inherited by the organism's progeny.
   B. skin or bone marrow cells which can be inherited by this organism's progeny.
   C. sperm or egg cells which cannot be inherited by the organism's progeny.
   D. skin or bone marrow cells which cannot be inherited by the organism's progeny.

4. Little research is being conducted on germ cell gene therapy because

   A. it is presently technologically impossible, although ethically acceptable.
   B. it is presently ethically unacceptable, although technologically possible.
   C. both ethical and technical problems surrounding its use have not been resolved.
   D. many of the ethical and technical problems surrounding its use have been resolved and no further research is necessary.

5. A physical method for introducing into a cell the DNA coding for a normal gene is

   A. electroporation.
   B. retrovirus carriers.
   C. calcium phosphate-mediated DNA uptake.
   D. restriction endonucleases.
6. Calcium phosphate-mediated DNA uptake is also called
   A. transduction.
   B. transformation.
   C. transplantation.
   D. transfection.

7. The method used for successfully introducing DNA for the rat growth hormone into mouse germ cells is
   A. electroporation.
   B. microinjection.
   C. retrovirus carriers.
   D. restriction endonucleases.

8. The method most likely to be used for human gene therapy is
   A. electroporation.
   B. calcium phosphate-mediated DNA uptake.
   C. retrovirus carriers.
   D. microinjection.

9. One advantage of using a retrovirus carrier is that it
   A. inserts many copies of the normal gene in one cell.
   B. prevents the cell from reproducing uncontrollably and causing cancer.
   C. is lost from the cell after repeated replications.
   D. can introduce the gene into a large number of cells with almost 100% efficiency.

10. Retroviruses can insert their genetic material into a cell’s genome by converting their
    A. RNA to DNA.
    B. DNA to RNA.
    C. RNA to proteins.
    D. DNA to proteins.

11. The role of the "helper" virus is to help the retrovirus carrying the human gene by
    A. supplying it with a protein coat.
    B. supplying it with an assembly signal.
    C. introducing it into the DNA of the patient’s cells.
    D. introducing the "normal" human gene into the carrier virus DNA.

12. The DNA which codes for the normal human gene is placed in the retrovirus' genetic material using
    A. restriction endonuclease.
    B. DNA polymerase
    C. transfection.
    D. deoxyribonuclease.
13. The retrovirus carrying the human gene is specially modified so that it
A. can infect any cell with which it comes in contact.
B. can reproduce uncontrollably.
C. cannot infect any cell with which it comes in contact.
D. cannot infect a second cell after it has entered one cell.

14. When the "corrected" cells are returned to the patient, it is hoped that they will
A. transfer their new, normal gene to nearby cells.
B. excrete their new, normal gene into the blood so that it can reach other parts of the body.
C. have a reproductive advantage and will replace the cells having the defect.
D. absorb the protein product of the defective gene.

15. Gene therapy has the immediate potential for helping persons having
A. genetic diseases involving chromosome inversions.
B. genetic diseases involving chromosome translocations.
C. genetic diseases resulting from recessively inherited, single gene disorders.
D. genetic diseases resulting from dominantly inherited, single gene disorders.

16. Ideal candidates for human gene therapy are
A. hemophilia and thalassemia.
B. ADA and PNP.
C. hormonal disorders.
D. skin cancer and leukemia.

17. Bone marrow cells are used for gene therapy because they
A. can be extracted relatively easily with a needle and syringe.
B. contain many stem cells which are capable of destroying cells with defective or nonfunctional genes.
C. have no genetic material and easily accept the inserted DNA.
D. are identical in all organisms and can be easily transplanted.

18. Some problems with gene therapy include
A. the inactivation of one of the individual's normal genes or the activation of a cancer-promoting gene.
B. the loss or migration of the "helper" virus gene within the patient's genome.
C. both a and b.
D. none of the above.
19. Possible long term effects on the organism after gene therapy include
   A. slowed growth.
   B. increased risk of cancer.
   C. both a and b.
   D. neither a nor b.

20. Presently, guidelines for researchers who wish to use human subjects in gene therapy experiments have
   A. not been approved, although NIH-sanctioned experiments on human somatic and germ cells have already occurred.
   B. been approved and NIH-sanctioned experiments on human somatic cells have occurred.
   C. been approved and NIH-sanctioned experiments on human germ cells have occurred.
   D. been approved, but neither NIH-sanctioned experiments on human somatic or germ cells has occurred.
GENE THERAPY OBJECTIVES

1. Identify a proper definition of "gene therapy" and its goals.

2. Compare and contrast somatic cell gene therapy and germ cell gene therapy.

3. Identify three (3) methods for introducing foreign DNA into cells having a nonfunctional or defective gene.

4. List several of the advantages of using viral carriers to introduce a normal gene into a cell having a nonfunctional or defective gene.

5. Identify the function of restriction endonucleases in the gene therapy process.

6. Describe the role of bone marrow cells and plasmids in human gene therapy.

7. Describe the function of retroviruses in human gene therapy.

8. List two or three human genetic defects that appear to be viable candidates for human gene therapy.

9. List some of the possible problems that could arise in human gene therapy.

10. Describe some precautions taken to prevent the hasty use of gene therapy without sufficient background research.
Gene therapy is a promising new method for treating severe genetic diseases. Gene therapy has as its goal "curing" a genetic disease by inserting the DNA of a normal gene into the cells of an organism with a defective or nonfunctional gene. Hopefully, the cells with the "corrected" set of genes will produce an adequate amount of the gene product in order to compensate for the cells with the defective or nonfunctional genes.

Gene therapy may involve either somatic or germ cells. Somatic cell gene therapy is concerned with the addition of normal genes to body cells such as bone marrow and skin cells. The changes made in the body cells cannot be inherited by the organism's progeny. Somatic cell gene therapy is presently the subject of numerous research projects. Germ cell gene therapy involves the addition of normal genes to cells which will eventually become sperm or egg cells. Any changes made to germ cells may be expected to be inherited by future generations. Little research has been carried out in this germ cell gene therapy due to many technical and ethical problems.

Currently there are three methods for introducing DNA which codes for normal gene products into cells with an abnormal gene. Microinjection and electroporation are physical methods of gene insertion. In MICROINJECTION, the DNA coding for the normal gene product is injected into each cell individually using a needle and syringe. This procedure is not very practical since it is extremely time-consuming and inefficient. Microinjection has been used successfully, however, in certain gene therapy experiments in mice.
The cloned gene for rat growth hormone, for example, has been injected into mouse zygotes which incorporated the rat gene into their own genetic material and grew to be twice the size of normal mice. Furthermore, these altered mice successfully transmitted the rat growth gene to their offspring. An electric current is used in ELECTROPORATION to move the DNA coding for the normal gene product directly across the cell membrane. The efficiency of this method has yet to be determined.

The chemical method of DNA insertion is calcium phosphate-mediated DNA uptake. Calcium phosphate is complexed with the DNA coding for the normal gene product and incubated with the cells containing a defective or nonfunctional gene. The calcium phosphate facilitates the entry of the DNA into the cells in a process called TRANSFECTION. Even with the calcium phosphate, however, transfection occurs only in approximately 1 in 1,000,000 cells. The large number of cells required to ensure an adequate number of "corrected" cells for this method makes it impractical for use in human gene therapy.

The method most likely to be used for human gene therapy is virus carriers of human genes. Viruses are ideal carriers of genetic material for many reasons. Viruses can introduce the genetic material into large numbers of cells with nearly 100% efficiency. Viruses only insert one copy of the normal gene into a cell, whereas other methods often result in several copies of the gene being inserted into the cell. Viral infection does not seem to affect permanently the cell's ability to reproduce. In addition, virally introduced genes remain integrated into the cell's genome for a longer period of time without migrating within the genome or being lost during replication.

Using a virus as a gene carrier to introduce a gene into a cell
with a genetic defect is a complicated process (see Figure 1). Retroviruses are presently the most commonly used viruses for gene therapy. Retroviruses are capable of converting their RNA into DNA and inserting it into a cell's genome. The DNA of the retrovirus, when integrated into the cell's genome, is called a provirus. In gene therapy, the proviral DNA is isolated from the cell's DNA and inserted into a bacterial plasmid. A PLASMID is a small circle of cytoplasmic DNA.

At this point, some of the retroviral genes are removed and replaced with the desired human gene. As in all methods, the human gene to be "corrected" must first be isolated in its normal form and its base pair sequence must be determined. The procedures for isolating and sequencing specific genes have become standard laboratory technique only recently. The base pair sequence which codes for the normal human gene product is placed in the retroviruses' genetic material using enzymes called restriction endonucleases. RESTRICTION ENDONUCLEASES clip the genetic material of the retrovirus at specific locations. When the base pair sequence coding for the normal human gene is clipped from a human chromosome using the same restriction endonuclease, the human gene can be inserted into the genetic material of the retrovirus. The retroviruses used to carry the human genes are specially modified so that they are incapable of entering a second cell after they have infected one cell. This modified virus no longer contains the gene needed to produce the protein coat that encases the viral genome.

The plasmid containing the retroviral and human DNA can be extracted from the bacteria and introduced into a special cultured cell line containing the DNA of a "helper" virus. The "helper" viral DNA
contains the genes necessary to produce a protein coat, but the gene which signals the assembly of the virus has been deleted. In order to reproduce, the retroviral DNA containing the human gene uses the protein coat produced by the "helper" viral DNA and its own signal for assembly. The retrovirus produced in this manner can infect one cell, but it cannot reproduce and leave that cell to infect other cells. The "helper" viral DNA cannot reproduce by using the assembly signal of the viral DNA carrying the human gene. Finally, retroviruses carrying the human gene are isolated and incubated with the cells removed from a patient with a defective or nonfunctional gene. The retroviruses inject their genetic material into the cells where it eventually is incorporated into the cells' DNA. The "corrected" cells are then returned to the patient with the hope that they will have a reproductive advantage over the cells with the defective genes. If these "corrected" cells have an advantage, they will replace the cells having the defect.

Although the promises of gene therapy are exciting, its use may be severely limited. Gene therapy is not applicable to genetic diseases involving large segments of chromosomes, polygenic inheritance, or environmentally-induced diseases. In addition, few of the more than 2,000 single gene disorders that have been identified may be helped. Single gene disorders of the blood, such as hemophilia and thalassemia, were first thought to be ideal candidates for gene therapy, but have since proven to be very difficult with which to work. Scientists are hampered in their efforts by the fact that the regulation of the hemoglobin genes is not fully understood and that many base pair substitutions may produce disease. Genetic diseases involving altered hormone production or function are not subjects for gene therapy for
many of the same reasons that make blood disorders impractical subjects. In addition, hormone disorders can be treated more safely, and with less difficulty, using hormones produced in bacteria by recombinant DNA technology.

The best candidates for correction by gene therapy with our present level of technology are relatively rare, recessively inherited, single gene disorders which cause the loss of a single enzyme (see Table 1). Diseases such as adenosine deaminase deficiency (ADA) and purine nucleoside phosphorylase deficiency (PNP) are likely to be the first single gene disorders for which human gene therapy is attempted. ADA occurs in only 40-50 births worldwide per year and PNP is even rarer occurring in only 8-10 births worldwide per year. Both diseases result in the lack of an immune system due to the absence of a single enzyme. The production of even a small amount of the appropriate enzyme for either disease would noticeably reduce the effects of the disease. A mild overproduction of the appropriate enzyme would not be likely to produce a harmful side effect. This is a necessary requirement, since the inserted gene may have been incorporated several times into one cell. ADA and PNP are suited particularly well for gene therapy since the cells which produce the enzyme are derived from the bone marrow. Bone marrow cells can be extracted from an individual with relative ease using a needle and syringe. Unfortunately, only approximately 0.01% of the bone marrow is composed of stem cells which are the cells capable of differentiating into the cells of the immune system which produce the enzyme.

Many important questions concerning gene therapy must still be answered before human gene therapy begins. Inserted genes have been shown to migrate within the individual’s genome or to be lost after
repeated replications. The possibility exists that one of the individual's normal genes will be inactivated or that an oncogene will be activated by the insertion of a new gene. The modified viral DNA inserted with the normal human gene may combine with undetected viral DNA in the cells. This could allow the virus to regain its ability to leave the cell and infect neighboring ones. Gene regulation has also proven to be extremely difficult since the insertion of the normal gene at a specific site cannot be controlled. The long term effects on an organism, such as slowed growth, reduced fertility, increased risk of cancer and early death are just beginning to be examined.

To prevent hasty use of gene therapy without sufficient background research, the Human Gene Therapy Working Group, a subcommittee of the federal Recombinant Advisory Committee (RAC), produced a pamphlet in 1985 outlining "Points to Consider in the Design and Submission of Human Gene Therapy Protocols." The working group which consists of scientists, ethicists, physicians, lawyers, public policy specialists, and a layperson developed guidelines for researchers who wish to use human subjects in gene therapy experiments. The guidelines suggest in vitro studies with human bone marrow followed by in vivo studies with mice and concluded with extensive in vivo experiments with primates before human gene therapy is attempted. Questions concerning previous research results, patient consent, patient selection, and publicity are asked as well. These questions must be answered satisfactorily before a local institutional review board, the working group, a full meeting of the RAC and the director of the National Institutes of Health (NIH) prior to any gene therapy involving human beings. Presently, no human gene therapy sanctioned by the NIH has been conducted, although many researchers have been able to insert new genes into the somatic cells
of mice using gene therapy techniques.
REFERENCES


*Especially useful paper.
Table 1. Enzyme deficiency diseases that are likely candidates for human gene therapy.

<table>
<thead>
<tr>
<th>Candidates for Human Gene Therapy</th>
<th>Number of Births Worldwide Per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase deficiency (Severe Combined Immune Deficiency)</td>
<td>40 - 50</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase deficiency</td>
<td>8 - 10</td>
</tr>
<tr>
<td>Arginosuccinate synthetase deficiency</td>
<td>53</td>
</tr>
<tr>
<td>Carbamoyl transferase deficiency</td>
<td>110</td>
</tr>
<tr>
<td>Glucose cerebrosidase deficiency (Gaucher's Disease)</td>
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</tr>
<tr>
<td>Mucopolysaccharidosis</td>
<td>--</td>
</tr>
<tr>
<td>Hypoxanthine-guanine phosphoribosyltransferase deficiency (Lesch-Nyhan Syndrome)</td>
<td>200</td>
</tr>
</tbody>
</table>
The retrovirus inserts its RNA into a cell where the RNA is converted into DNA and incorporated into the cell's genome.

1. The retrovirus DNA is isolated and inserted into a bacterial plasmid.

2. The DNA coding for the normal human gene is used to replace some of the retrovirus genes.

3. The plasmid multiplies and is isolated.

4. The plasmid is inserted into a cell containing "helper" virus DNA.

5. The "helper" virus DNA produces a protein coat.

6. The carrier retrovirus uses the protein coats produced by the "helper" virus DNA to reproduce.

7. The carrier retroviruses are isolated.

8. The viruses are incubated with the patient's cells, allowing the human and viral genes to be incorporated into the patient's genome.

9. The "corrected" cells are returned to the patient.
BIO 199
QUESTIONS TO CONSIDER CONCERNING GENE THERAPY

1. What do you think are some of the possible consequences of germ cell gene therapy?

2. Do you think that the risks of gene therapy are worth its potential benefits? How would you assess these risks?

3. Who do you think ought to have the power to decide what diseases or traits will be "corrected" by gene therapy?

4. Do you think that today's society ought to be allowed to direct the evolution of future generations through the use of gene therapy?

5. Do you think gene therapy ought to be considered "playing God"?

6. Do you think that our society ought to encourage gene therapy over more conventional techniques for treating genetic diseases?
BIO 199
FOLLOW-UP QUESTIONS FOR DISCUSSION LEADERS

1. What do you think are some of the possible consequences of germ cell gene therapy?
   a. What diseases or traits do you think ought to be "corrected" by gene therapy?
   b. What diseases or traits do you think ought to receive priority treatment?
   c. Do you think a new demand for eugenics (the selected breeding of individuals in order to improve the condition and capabilities of the human race) will arise?
   d. Do you think we could inadvertently remove those qualities (e.g., music appreciation, art appreciation) that make us uniquely human?

2. Given the following data, do you think the risks of gene therapy are worth its potential benefits? How would you assess these risks?
   a. Presently scientists cannot regulate 1) the place of insertion of the gene, 2) the inactivation of the person's normal genes, and 3) the activation of oncogenes.
   b. The possible effects on germ cells have not been determined.
   c. The effects on the gene pool have not been determined.

3. Who do you think ought to have the power to decide what diseases or traits will be "corrected" by gene therapy?
   b. Ought allowances be made for cultural differences?
   c. Assuming that there is a hereditary factor involved in certain behaviors (i.e., aggression, sexual deviancy, etc.), ought these traits be "corrected" using gene therapy?
   d. What ought to be done with someone who refuses treatment for a condition determined to be undesirable?

4. Do you think that today's society ought to be allowed to direct the evolution of future generations through the use of gene therapy?
   a. Does today's society have an obligation to protect future generations from harm?
   b. Is gene therapy a form of human experimentation on future generations without their approval? Is their approval an important consideration?

5. Do you think gene therapy ought to be considered "playing God"?

6. Do you think that our society ought to encourage gene therapy over more conventional techniques for treating genetic diseases?
   a. Ought money be diverted from less glamorous health care measures to pay for gene therapy?
   b. Joseph Fletcher, an ethicist, promotes the use of germ cell gene therapy because somatic cell gene therapy allows its patients to pass on their "bad genes" to their offspring. Ought that be a consideration?
BIO 199  
GENE THERAPY DISCUSSION EVALUATION  

Either check the appropriate response, or if you prefer, reply in the space provided.  
DO NOT SIGN YOUR NAME.  

1. Do you feel that the discussion moved satisfactorily towards a solution to the problem?  
   YES _____ NO _____ A solution was reached, but not through a logical process. _____  

2. Did the discussion cause you to look at the problem from a viewpoint other than your own?  
   YES _____ NO _____ Undecided _____  

3. Were you permitted to express your views without hostility or ridicule from others?  
   YES _____ NO _____ Undecided _____  

4. Did the reading material provide you with enough background information to discuss intelligently the potential use of human gene therapy?  
   YES _____ NO _____ Undecided _____  

5. Ought somatic cell gene therapy be attempted on human beings?  
   YES _____ NO _____ Undecided _____  

6. Ought germ cell gene therapy be attempted on human beings?  
   YES _____ NO _____ Undecided _____  

7. Do you feel that the discussion was a profitable use of your time?  
   YES _____ NO _____ Undecided _____  

8. Did the discussion help you form an opinion on the issue?  
   YES _____ NO _____ Undecided _____  

9. Name of your discussion leader:__________________________________________  

10. Please list any suggestions you may have to improve the discussion or the reading material.
1. Ideal candidates for human gene therapy are
   A. hemophilia and thalassemia.
   B. ADA and PNP.
   C. hormonal disorders.
   D. skin cancer and leukemia.

2. The retrovirus carrying the human gene is specially modified so that it
   A. can infect any cell with which it comes in contact.
   B. can reproduce uncontrollably.
   C. cannot infect any cell with which it comes in contact.
   D. cannot infect a second cell after it has entered one cell.

3. Retroviruses can insert their genetic material into a cell’s genome by converting their
   A. RNA to DNA.
   B. DNA to RNA.
   C. RNA to proteins.
   D. DNA to proteins.

4. The method used for successfully introducing DNA for the rat growth hormone into mouse germ cells is
   A. electroporation.
   B. microinjection.
   C. retrovirus carriers.
   D. restriction endonucleases

5. Little research is being conducted on germ cell gene therapy because
   A. it is presently technologically impossible, although ethically acceptable.
   B. it is presently ethically unacceptable, although technologically possible.
   C. both ethical and technical problems surrounding its use have not been resolved.
   D. many of the ethical and technical problems surrounding its use have been resolved and no further research is necessary.

6. The goal of gene therapy is "to cure" a genetic disease
   A. temporarily.
   B. by administering hormones produced in bacteria by recombinant DNA technology.
   C. involving either large segments of chromosomes, polygenic inheritance, or environmentally-induced disease.
   D. permanently.

7. Possible long term effects on the organism after gene therapy include
   A. slowed growth.
   B. increased risk of cancer.
   C. both a and b.
   D. neither a nor b.
8. The role of the "helper" virus is to help the retrovirus carrying the human gene by
   A. supplying it with a protein coat.
   B. supplying it with an assembly signal.
   C. introducing it into the DNA of the patient's cells.
   D. introducing the "normal" human gene into the carrier virus DNA.

9. Bone marrow cells are used for gene therapy because they
   A. can be extracted relatively easily with a needle and syringe.
   B. contain many stem cells which are capable of destroying cells with defective or nonfunctional genes.
   C. have no genetic material and easily accept the inserted DNA.
   D. are identical in all organisms and can be easily transplanted.

10. When the "corrected" cells are returned to the patient, it is hoped that they will
    A. transfer their new, normal gene to nearby cells.
    B. excrete their new, normal gene into the blood so that it can reach other parts of the body.
    C. have a reproductive advantage and will replace the cells having the defect.
    D. absorb the protein product of the defective gene.

11. The method most likely to be used for human gene therapy is
    A. electroporation.
    B. calcium phosphate-mediated DNA uptake.
    C. retrovirus carriers.
    D. microinjection.

12. A physical method for introducing into a cell the DNA coding for a normal gene is
    A. electroporation.
    B. retrovirus carriers.
    C. calcium phosphate-mediated DNA uptake.
    D. restriction endonucleases.

13. The DNA of cells is "corrected" for use in gene therapy by
    A. deleting a defective or nonfunctional gene from the organism's cells.
    B. supplying to the cells hormones produced in bacteria by recombinant DNA technology.
    C. inserting the DNA of a normal gene into the cells of an organism with a defective or nonfunctional gene.
    D. removing the cells containing the defective or nonfunctional gene from the organism.
14. Presently, guidelines for researchers who wish to use human subjects in gene therapy experiments have
   A. not been approved, although NIH-sanctioned experiments on human somatic and germ cells have already occurred.
   B. been approved and NIH-sanctioned experiments on human somatic cells have occurred.
   C. been approved and NIH-sanctioned experiments on human germ cells have occurred.
   D. been approved, but neither NIH-sanctioned experiments on human somatic or germ cells has occurred.

15. Some problems with gene therapy include
   A. the inactivation of one of the individual's normal genes or the activation of a cancer-promoting gene.
   B. the loss or migration of the "helper" virus gene within the patient's genome.
   C. both a and b.
   D. none of the above.

16. Gene therapy has the immediate potential for helping persons having
   A. genetic diseases involving chromosome inversions.
   B. genetic diseases involving chromosome translocations.
   C. genetic diseases resulting from recessively inherited, single gene disorders.
   D. genetic diseases resulting from dominantly inherited, single gene disorders.

17. The DNA which codes for the normal human gene is placed in the retrovirus' genetic material using
   A. restriction endonuclease.
   B. DNA polymerase.
   C. transfection.
   D. deoxyribonuclease.

18. One advantage of using a retrovirus carrier is that it
   A. inserts many copies of the normal gene in one cell.
   B. prevents the cell from reproducing uncontrollably and causing cancer.
   C. is lost from the cell after repeated replications.
   D. can introduce the gene into a large number of cells with almost 100% efficiency.

19. Calcium phosphate-mediated DNA uptake is also called
   A. transduction.
   B. transformation.
   C. transplantation.
   D. transfection.
20. Somatic cell gene therapy involves changes in

A. sperm or egg cells which can be inherited by the organism's progeny.
B. skin or bone marrow cells which can be inherited by this organism's progeny.
C. sperm or egg cells which cannot be inherited by the organism's progeny.
D. skin or bone marrow cells which cannot be inherited by the organism's progeny.
PRENATAL DIAGNOSIS OBJECTIVES

1. Distinguish between the two general formats used in prenatal diagnosis.

2. Describe several methods used in performing laboratory analyses of fetal cells or proteins.

3. Identify reasons why misdiagnoses are a serious concern in prenatal diagnosis.

4. Outline four methods for the collection of fetal cells or proteins.

5. Describe the advantages and disadvantages of amniocentesis.

6. Explain why CVS is gaining in popularity.

7. Discuss situations in which CVS ought not to be performed.

8. Explain how the age of the fetus is accurately determined and why it is important in CVS.

9. Explain why there is an increased risk of misdiagnosis in CVS relative to other methods of prenatal diagnosis.

10. Describe several of the complications associated with CVS.
Prenatal Diagnosis

In recent years, medical science's ability to produce quick, reliable diagnoses has greatly increased. Nowhere has this increase been so dramatic as in the area of prenatal diagnosis. Literally hundreds of diseases and abnormalities can now be diagnosed while the fetus is still in the uterus, thus facilitating in utero treatment of the fetus, if possible, or an abortion if desired.

Formats of Prenatal Diagnosis

Currently, there are two general formats used in prenatal diagnosis: fetal visualization and fetal cell studies. Techniques such as radiology, fetoscopy, ultrasonography and magnetic resonance imaging (MRI) can be used to visualize the fetus directly and observe possible defects. Secondly, laboratory tests involving cytogenetic techniques, DNA technology, and radioimmunoassays can be used to diagnose fetal diseases using cells collected from the fetus.

Direct visualization of the fetus is useful in detecting structural abnormalities. Radiography, which involves the observation of the fetus using X-rays, and fetoscopy, which involves the use of optical fiber technology, have largely been replaced by ultrasound and MRI. Ultrasonography, the most widely used technique, constructs an image of the fetus using high frequency, low intensity soundwaves which are either absorbed by tissue or reflected back to the instrument. A computer uses this information to produce the fetal image on a monitor. MRI works in a similar manner using magnets and radiowaves. In addition to providing an image of high resolution (even the upper lip...
of the fetus can be distinguished!), ultrasound and MRI avoid the hazards to the fetus associated with X-ray radiation and allow physicians to view large sections of the fetus at once. Both ultrasound and MRI are used in conjunction with techniques to collect fetal cells for laboratory analysis.

Laboratory analysis of fetal cells or fetus-produced proteins can also be used to diagnose disorders prenatally. Chromosomal abnormalities can be identified by growing fetal cells in culture, stopping their growth and disrupting their nuclei. The cells' chromosomes are then strained, photographed and arranged according to size and banding patterns into a karyotype which allows the physician to identify chromosomal abnormalities.

Recent advances in DNA technology have introduced the use of restriction fragment length polymorphisms (RFLPs) to detect disorders prenatally. In this technique, restriction enzymes are used to clip DNA at specific sites. When mutations, insertions or deletions are introduced into a normal sequence of DNA, the sites clipped by the enzyme are altered. As a result, the restriction enzymes clip new DNA fragments of different lengths, making it possible to distinguish between the normal gene and a mutant one. Short, radioactive sequences of DNA (probes) which are complementary to specific genes are used to identify deletions or insertions in specific genes.

Biochemical tests look for the presence or absence of specific proteins to indicate such conditions as ectopic (outside the uterus) pregnancies, fetal lung immaturity and neural tube defects (NTDs). For instance, elevated levels of alpha-fetoprotein (AFP) are associated with neural tube defects which occur in approximately two in 1,000 live births. The amount of AFP is measured using a radioimmunoassay.
radioimmunoassay, the amount of radioactive antibodies which bind to a particular protein is used to calculate the concentration of the protein.

Prenatal diagnosis is not foolproof. Misdiagnosis of the fetus' condition is a serious concern with all forms of prenatal diagnosis. Although rare, errors in sex determination, chromosome number, and biochemical analysis have been reported. Some suggest that any risk of misdiagnosis is unacceptable because it may lead to the abortion of a genetically healthy child or the birth of an affected baby to parents expecting a healthy child.

Methods for Collection of Fetal Cells and/or Proteins

Maternal Blood Sampling. Some diseases can be detected using fetal cells or proteins collected from maternal blood. Accurate levels of AFP, for example, can be determined in this manner. In addition, cells that have migrated from the fetus into the mother's blood can be radioactively labeled and separated from the mother's blood using a fluorescent-activated cell sorter. Currently, this technique is only used to determine the presence of Y chromatin, but it is hoped that as the procedure improves enough cells will be collected for additional chromosomal and biochemical tests.

Fetal Blood Sampling. Fetal blood samples can be withdrawn from the placenta or umbilical cord using a needle guided by ultrasonography or fetoscopy for diagnosis of blood diseases, immune system deficiencies, metabolic diseases, and infections. Samples of tissue from fetal organs such as the skin and liver have also been successfully collected for analysis in much the same manner. Both blood and tissue removal may be hazardous to the fetus, however, and
may result in fetal loss.

**Amniocentesis.** Presently, most fetal cells and proteins are collected for analysis using amniocentesis. Amniocentesis involves the removal of 40 milliliters of fluid, which surrounds and cushions the fetus, from the amniotic sac. The amniotic fluid contains a few live cells sloughed off the fetus, many dead fetal cells and several proteins produced by the fetus. The fluid is removed using a needle inserted through the mother's abdominal wall and into the uterus. Since relatively few cells are collected, the live cells must be grown (cultured) for up to four weeks before some types of chromosomal, genetic, and biochemical analyses can be done. Amniocentesis is a relatively safe procedure if done in conjunction with ultrasonography, inducing fetal loss in only one in two hundred patients. This risk of fetal loss is approximately the same as that for women in the same stage of pregnancy who have not had amniocentesis. The major disadvantage of amniocentesis is that it does not enable a diagnosis until the second trimester of pregnancy, which increases the risk to the woman's health if she chooses an abortion based on the results of the amniocentesis.

**Chorionic Villi Sampling.** A promising technique of prenatal diagnosis which may one day replace amniocentesis is chorionic villi sampling (CVS). CVS relies upon the analysis of fetal cells removed from the chorionic villi (fingerlike projections of the membrane that surrounds the early embryo) for diagnosis of fetal disorders. Developed in the early 1960s by Swedish and Danish researchers, CVS was overshadowed by the more popular technique of amniocentesis. CVS is presently used in such countries as China, France, Great Britain, and the Soviet Union. In 1983, the United States approved CVS for
The recent rise in popularity of CVS stems from its ability to provide a diagnosis six weeks earlier in pregnancy than amniocentesis can. Results of chorionic villi sampling are usually obtained by the eleventh week of pregnancy, whereas amniocentesis cannot even be performed until the sixteenth week of pregnancy. The earlier diagnosis allows the woman to obtain a first trimester abortion if she chooses to do so. A first trimester abortion is both physically and emotionally better for the woman because it can be performed on an outpatient basis with less danger to the woman's health. It has also been suggested that a first trimester abortion is emotionally easier for the woman because the presence of the fetus is less obvious.

CVS was first used in the 1970s as a quick method for determining fetal sex, since the cells can be obtained quickly and do not require culturing before analysis. Today CVS is used to obtain cells for diagnosis of X-linked recessive disorders (hemophilia and muscular dystrophy), autosomal recessive diseases (Tay-Sachs, thalassemias, and sickle cell anemia), and chromosomal disorders such as trisomy-21. Cells obtained by CVS cannot be used to detect neural tube defects, however, because levels of alpha-fetoprotein necessary for diagnosis are found only in the amniotic fluid which surrounds the fetus or in the maternal blood.

Samples of chorionic villi can be obtained in three ways. The first tissue samples were removed using a needle which was inserted through the abdominal wall and uterus and was guided to the chorionic villi using fetoscopy. Chorionic villi biopsy (CVB) obtains samples of the chorionic villi by the use of biopsy forceps which pluck small pieces of tissue. The most common method of CVS, however, involves the
aspiration of small pieces of the chorionic villi using a syringe attached to a catheter inserted through the vagina.

The process of CVS begins with the collection of a thorough medical history from the patient. Women who have a history of bleeding, cervical polyps, benign tumors of the uterus or a sexually transmitted disease are discouraged from seeking CVS. Many physicians discourage the use of CVS on women with Rh-negative blood because of the possibility of causing the Rh-negative mother to produce antibodies to the Rh-positive blood of her fetus. The antibodies in the mother's blood pass through the placenta and destroy the fetus' blood unless drastic action is taken.

Ultrasound scanning is then used to determine the viability of the pregnancy and the practicality of performing CVS. From the ultrasound image, the physician can monitor fetal movements and heart rate. If twins are indicated, the woman is discouraged from having CVS due to the difficulty of obtaining the tissue samples without maternal cell contamination or damage to the fetuses. Using ultrasound, the physician can determine the position of the uterus, the insertion site of the umbilical cord and the location of the developing placenta. A short cervix or poorly positioned uterus may cause "shadows" cast by the surgical instruments to obstruct the physician's view of the uterus. If the ultrasound image shows that the developing placenta is attached to the rear of the uterus, the physician can prepare for the more difficult task of removing a tissue sample without infection or damage to the fetus.

The most important information gained from the ultrasound examination is the age of the fetus. This is determined by measuring the length of the fetus' upper leg (femur) or the diameter of the
widest part of the head. Accurate determination of fetal age is necessary because sampling must occur nine to ten weeks from the date of the last menstrual period of the woman. The chorionic villi only begin to develop approximately two weeks after conception and are sufficiently mature for sampling only after six weeks of gestation. Approximately ten weeks after conception, part of the chorion develops into the placenta while the rest of the chorion becomes a thin membrane lining the amnion. CVS must, therefore, be performed within a narrow time frame, dependent upon the age of the fetus. Many women are referred too late for CVS due to the miscalculation of the date of conception.

In the most common method of CVS, the internal and external genitalia of the woman, as well as the surgical instruments, are covered with an antibiotic. A tenaculum (a hooked surgical instrument) is used to straighten and hold open the cervix, as a plastic catheter is passed through the vagina and into the uterus (see Figure 1). Ultrasound imaging is used to guide the catheter to the chorionic villi. Approximately 30 mg of tissue are suctioned from the end of one or more villi using a syringe attached to the end of the catheter. The procedure, which is relatively painless, is usually performed in less than 45 minutes on an outpatient basis with no anesthesia. The patient usually returns two to four days later for a third ultrasound examination to reassure the physician and the patient of the viability of the fetus.

The tissue collected by CVS is immediately examined after removal from the patient. The syncytial buds, which contain many nuclei, are separated from the other cells of the chorion and prepared for direct observation for chromosomal or biochemical changes. Often
preliminary results can be obtained within 24 hours of CVS. The quality of the karyotype from the direct observation of chorionic villi cells is poorer, however, than a karyotype of cells obtained by amniocentesis, increasing the risk of misdiagnosis. Tissues of the chorion commonly contain a few cell lines with chromosomal abnormalities that are not indicative of the fetus' condition. These cells, which are selectively eliminated later in pregnancy, can produce an ambiguous diagnosis. Many physicians prefer to confirm the results of preliminary tests by repeating them on the fetal cells after a short-term (3 to 10 days) tissue culture.

Complications have been associated with the use of CVS. Some women have experienced excessive bleeding or spotting after CVS. The bleeding or spotting usually subsides after 72 hours, apparently causing no harm. Many physicians believe that the heat generated by the ultrasound waves may damage fetal cells and is capable of causing slowed prenatal growth, immune system abnormalities, hearing problems and other developmental defects due to the immaturity of the fetus' organs. Abnormalities have been found in some fetuses after CVS with the use of ultrasound, but the cause of the abnormalities could not be specifically determined. Some early discussions of CVS reported the inadvertent puncturing of the amniotic sac resulting in fetal loss. The incidence of fetal loss has decreased, however, as physicians have improved their ability to visualize the two-dimensional image of the ultrasound picture as the three-dimensional structure of the uterus.

Bacterial infection of the developing fetus is also a major concern. Several potentially disease-causing bacteria have been isolated from the catheter used on the patient and from the patient's
cervix. Most physicians limit the number of attempts made to collect an uncontaminated sample in order to lessen the possibility of infection. Contamination of the tissue sample may be from either bacteria or maternal cells. Both types of contamination can prevent the proper evaluation of the fetal cells.

The most significant complication associated with CVS appears to be an increased risk of miscarriage. Worldwide, there is a 2-5.6% rate of fetal loss following CVS. Although some fetal losses have resulted from contractions induced during CVS, it has been suggested that the apparently increased numbers of miscarriage may be a result of the time at which the procedure is performed rather than the actual procedure. The fetus, some suggest, may be experiencing a sensitive growth period during which miscarriages are more common at the time CVS is performed. See Table 1 for a comparison of three methods for obtaining fetal cells.

Prenatal diagnosis is a rapidly developing field. New techniques have made it possible to detect many diseases in the fetus. Early diagnosis of fetal disorders has increased dramatically the choices available to parents who have an increased risk of producing a child with an inborn disease. As techniques for prenatal diagnosis improve, parents will benefit from the ability to prepare for fetal disorders.
REFERENCES


FIGURE 1

CHORIONIC VILLI SAMPLING

Ultrasound

Chorion

Fetus

Syringe

Catheter
Table 1: Comparison of Three Methods for Obtaining Fetal Cells

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<tr>
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<th>Amniocentesis</th>
<th>CVS</th>
<th>Fetal Blood Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normally Performed at:</td>
<td>16 Weeks</td>
<td>8-10 Weeks</td>
<td>18 Weeks</td>
</tr>
<tr>
<td>If Successful, Results Obtained in:</td>
<td>Chromosomal = 2 Weeks</td>
<td>Chromosomal = 24 Hours</td>
<td>Chromosomal = Several Days</td>
</tr>
<tr>
<td></td>
<td>Biochemical = 4 Weeks</td>
<td>Biochemical = 2 Weeks</td>
<td>Biochemical = Several Days</td>
</tr>
<tr>
<td>Contamination of Sample:</td>
<td>Rare</td>
<td>Maternal Cells</td>
<td>Amniotic Fluid</td>
</tr>
<tr>
<td>Estimated Risk of Inducing Spontaneous Abortion:</td>
<td>&lt;0.5%</td>
<td>R = 2.0-5.6%</td>
<td>R = 2.0-5.0%</td>
</tr>
<tr>
<td>Requires:</td>
<td>Ultrasound</td>
<td>Ultrasound</td>
<td>Ultrasound or Fetoscopy</td>
</tr>
<tr>
<td>Enables the Detection of NTD's:</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
1. Misdiagnosis of the fetus' condition after amniocentesis is a serious concern because it may
   a. result in extremely high malpractice insurance costs for the doctors.
   b. cause the procedure to be banned.
   c. lead to the abortion or treatment of a genetically healthy fetus.
   d. require a more dangerous diagnostic procedure to be performed.

2. The fetus can be directly visualized using
   a. cytogenetic techniques.
   b. radioimmunoassays.
   c. fetoscopy.
   d. all of the above.

3. By themselves, direct visualization techniques are used to detect
   a. physical abnormalities.
   b. Rh antigen–antibody reactions between the mother and fetus.
   c. the presence or absence of specific proteins.
   d. chromosomal abnormalities.

4. Biochemical tests look for
   a. changes in chromosome number.
   b. the presence or absence of specific proteins.
   c. varying lengths of DNA sequences.
   d. binding between radioactive DNA probes and the fetus' DNA.

5. RFLPs distinguish between normal and abnormal genes by
   a. the binding of radioactive probes to specific genes of the fetus.
   b. the arrangement of stained chromosomes into a karyotype.
   c. the difference in length of DNA fragments after clipping with restriction enzymes.
   d. the amount of radioactive antibodies which bind to the normal gene compared to the
      abnormal gene.

6. Presently, the most common method for the collection of fetal cells and proteins is
   a. maternal blood sampling.
   b. fetal blood sampling.
   c. amniocentesis.
   d. chorionic villi sampling.
7. An advantage of amniocentesis is
   a. the low rate of miscarriage associated with the procedure.
   b. the ability to prepare a karyotype directly from the collected cells.
   c. that it can be performed in the first trimester of pregnancy.
   d. that many rapidly growing cells are collected at one time.

8. Amniocentesis is preferred over CVS for the detection of neural tube defects because
   a. results can be obtained earlier in pregnancy since cells do not need to be cultured.
   b. high levels of proteins similar to AFP result in misdiagnosis.
   c. levels of AFP necessary for diagnosis are found in the amniotic fluid and not in the chorionic villi.
   d. live fetal cells are obtained in an earlier stage of growth.

9. Gestational age of the fetus is usually determined in CVS by measuring
   a. the amount of AFP present in amniotic fluid.
   b. the length of the femur or the diameter of the widest part of the head.
   c. the circumference of the mother’s waist.
   d. the number of days since the mother’s last menstrual period.

10. CVS must be performed within a narrow time frame dependent upon the fetus’ age because
     a. the chorionic villi do not develop fully until after several weeks of gestation and then they disappear.
     b. the fetus becomes too large for the insertion of surgical instruments into the uterus.
     c. the level of proteins necessary for prenatal diagnoses are no longer present after a certain age.
     d. cell lines with abnormal chromosome numbers not indicative of the fetus’ condition develop in the placenta after a certain age.

11. The possibility of misdiagnosis is increased in CVS over other methods of prenatal diagnosis because
     a. the cells grown in culture mutate over time.
     b. the preparation of the cells for karyotyping often damages the cells’ chromosomes.
     c. the placenta contains cell lines with chromosomal abnormalities that are not indicative of the fetus’ condition.
     d. few live cells undergoing mitosis are collected resulting in too few karyotypes to make a definite diagnosis.

12. To limit the possibility of misdiagnosis in CVS, doctors often grow the fetal cells
     a. in short term (3-10 days) culture.
     b. in long term (2-4 weeks) culture.
     c. in a nutrient broth which eliminates maternal cells.
     d. in a nutrient broth which eliminates abnormal cells.
13. Ultrasound is used with caution in prenatal diagnosis because it
   a. often shows abnormalities that do not exist.
   b. endangers the health of the mother.
   c. may introduce infection into the uterus.
   d. may generate enough heat to damage fetal cells.

14. Physicians attempt to limit the introduction of infection during CVS by
   a) limiting the number of attempts to collect an uncontaminated sample.
   b. giving the mother large quantities of antibiotics prior to CVS.
   c. using the same catheter for each attempt on a woman to collect a chorionic villi sample.
   d. allowing only the physician to be in the room during the procedure.

15. The increased risk of miscarriage associated with CVS may be the result of everything EXCEPT
   a. the induction of contractions during the procedure.
   b. the period of gestation during which the procedure is performed.
   c. the induction of minor bleeding or spotting during CVS.
   d. the puncturing of the amniotic sac during CVS.

16. The safest method for the fetus of collecting fetal cells is through
   a. maternal blood sampling.
   b. fetal blood sampling.
   c. amniocentesis.
   d. chorionic villi sampling.

17. The most significant reason for the rise in popularity of CVS is that it
   a. detects neural tube defects.
   b. can be performed in the first trimester of pregnancy.
   c. can be performed more safely than any other technique for the collection of fetal cells.
   d. decreases the risk of misdiagnosis.

18. Contraindications to CVS include everything EXCEPT
   a. twins.
   b. Rh incompatibility between mother and fetus.
   c. maternal history of sexually transmitted diseases.
   d. advanced maternal age.

19. The popularity of CVS will likely increase most dramatically if
   a. the rate of miscarriage decreases.
   b. the procedure can be performed earlier in pregnancy.
   c. the procedure can be performed in less time and with less pain.
   d. the number of living cells collected can be increased significantly.
20. Fetal blood sampling, amniocentesis, and CVS are safest when performed

a. before the sixth week of pregnancy.
b. on an outpatient basis at a hospital.
c. after the twelfth week of pregnancy.
d. in conjunction with ultrasonography.
BIO 199
PRENATAL DIAGNOSIS HANDOUT EVALUATION

Either check the appropriate response, or if you prefer, reply in the space provided.
DO NOT SIGN YOUR NAME.

1. How many times did you read or review the material?
   ___ 0 times  ___ 1-2 times  ___ 3-4 times  ___ 5 or more times

2. Was the reading material interesting?
   ___ YES  ___ NO  ___ Undecided

3. Was the reading material easy to understand?
   ___ YES  ___ NO  ___ Undecided

4. Did the reading material assist in your understanding of the lecture?
   ___ YES  ___ NO  ___ Undecided

5. Do you feel that the handout was a valuable addition to the lecture?
   ___ YES  ___ NO  ___ Undecided

6. Did the handout present new information to you?
   ___ YES  ___ NO  ___ Undecided

7. Do you feel that figure 1 (the cross section of CVS) was a helpful addition to the handout?
   ___ YES  ___ NO  ___ Undecided

8. Do you feel that table 1 (Comparison of Three Methods for Obtaining Fetal Cells) was a useful addition to the handout?
   ___ YES  ___ NO  ___ Undecided

9. Does the content of this handout suggest any ethical questions to you?
   ___ YES  ___ NO  ___ Undecided

10. Please list any suggestions you might have to improve the handout entitled "Prenatal Diagnosis".