Learning Objectives:
1. Learn to work in small groups effectively to solve a clinical problem.
2. Describe the how cystic fibrosis is diagnosed using a sweat test.
3. Describe the normal versus pathologic transport mechanisms of the CFTR channel.
4. Discuss the effect of an obstructive lung disorder on pulmonary mechanics.
5. Discuss the ethical issues surrounding children in medical research.
6. Describe how an SDS-PAGE gel works.
7. Discuss the inheritance pattern for cystic fibrosis and the likelihood of passing the gene or disease from one generation to another.

Case:
Mr. and Mrs. Gottleib took their nine-month-old infant, Jeremy, to the emergency room because he had been suffering from a cough and diarrhea for almost a week. They told the doctor that Jeremy would sometimes "wheeze" a lot more than they thought was normal for a child with "just a cold" which is what their pediatrician said the problem was last week.

Upon arriving at the emergency room, the attending pediatrician noted that salt crystals were present on Jeremy’s skin, and chest auscultation revealed abnormal sounds. The attending pediatrician ordered a chest x-ray and asked Jeremy’s parents to sit with him in his office to discuss the situation.

The pediatrician told the Gottleibs that Jeremy was small for his age, something he called "failure to thrive". He also mentioned the salt crystals on the baby’s skin, at which time Mr. Gottleib said "Jeremy has always had salty skin, I call him my ‘Little Pretzel Stick’ because his skin tastes salty when I kiss him." He said Jeremy’s symptoms all point toward a specific disease, and a sweat test would be needed to confirm his suspicions.

To do a sweat test, a small patch of skin on the child’s forearm is first cleaned. A gauze pad saturated with pilocarpine, a chemical that makes the skin sweat, is then applied to the area. Electrodes are hooked up, and a mild electric current is turned on for five minutes, which drives the pilocarpine into the skin. A sweaty area appears on the skin where the gauze had been placed, and a piece of dry filter paper is taped over it to absorb the sweat for about a half hour. A technician then measures the concentration of chloride in the pad.

The following day - "I'll never forget that day" - Jeremy’s mother says, the pediatrician called with the results of the sweat test. The doctor told Mrs. Gottleib that Jeremy’s chloride level was much higher than normal. This meant that the test was positive and revealed that the pediatrician’s suspicions were correct, Jeremy has cystic fibrosis. The pediatrician explained that Jeremy’s respiratory symptoms were caused by the cystic fibrosis because his airways were becoming distended by thick and tenacious mucus secretions which cannot be cleared from the airways in the normal fashion.

Jeremy’s Chest x-ray:
http://myweb.lsbu.ac.uk/dirt/museum/margaret/68--252-3041141.jpg
Questions:

1. Which of the following mechanisms most accurately explains how a mutation to the CFTR gene results in thicker than normal mucus secretions in the respiratory airways?

   A. Produces improper protein folding which prevents normal function of cilia.
   B. Reduces the number of CFTR proteins on the epithelial cell plasma membranes.
   C. Produces chloride transporters with a lower than normal Km.
   D. Causes improper splicing of the hnRNA.
   E. Increases the expression of aquaporin channels in bronchial epithelial cells.

2. As Jeremy’s disease progresses, he may develop bronchiectasis, an abnormal stretching and enlarging of the respiratory passages caused by mucus blockage, which is an obstructive lung disease. If this were to happen, predict the changes to the following respiratory parameters.

<table>
<thead>
<tr>
<th>Forced vital capacity (FVC)</th>
<th>Forced expiratory volume in 1 sec. (FEV₁)</th>
<th>FEV₁ / FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A increased</td>
<td>increased</td>
<td>80%</td>
</tr>
<tr>
<td>B decreased</td>
<td>decreased</td>
<td>40%</td>
</tr>
<tr>
<td>C decreased</td>
<td>decreased</td>
<td>80%</td>
</tr>
<tr>
<td>D normal</td>
<td>increased</td>
<td>90%</td>
</tr>
<tr>
<td>E decreased</td>
<td>Normal</td>
<td>90%</td>
</tr>
</tbody>
</table>

The pediatrician explained to Jeremy’s parents that research for cystic fibrosis is happening world wide to find a cure for the disease. He told them about a group of scientists doing medical research, and asked if they would allow Jeremy to be involved in a study. After a discussion about the nature of the research, Jeremy’s parents agreed allow him to participate in the study.

3. Which of the following is the correct protocol that must be applied when using children for medical research?

   A. The child between the ages of 7to17 years is required to give assent.
   B. Only one parent is required for consent.
   C. The parents can obtain some material benefit for having their child participate.
   D. Verbal information to the parent is adequate as informed consent.
   E. Verbal agreement by the parents is adequate for consent to the study.

The medical study which Jeremy was involved in included a pulse-chase experiment (figure below), using lung epithelial cells from a normal volunteer (A) and Jeremy (B). The cells were incubated for fifteen minutes in methionine-free media containing radioactive $^{35}$S-labeled methionine (the pulse). The radioactive methionine was then washed away, and the cells were incubated in medium containing an excess of unlabelled methionine for the indicated periods of time (the chase). At each time point, the cells were homogenized and an immunoprecipitation was performed using an antibody to CFTR. The immunoisolates were separated on an SDS-PAGE gel and the dried gel was exposed to x-ray film. The figure below shows a simplified drawing of the exposed x-ray film. On the right of each gel are shown the migration positions of molecular weight markers on the SDS-PAGE gel. *(This figure was drawn based on data shown in Lukacs et al., 1994.)*
4. The shift in the apparent molecular weight of CFTR seen in lanes 4 and 5 of gel A, but not gel B, is most likely due to

A. glycosylation of normal CFTR.
B. degradation of normal CFTR.
C. assembly of normal CFTR into a complex.

5. Ten years after Jeremy's diagnosis, the Gottlieb’s have had a daughter who is also affected with Cystic Fibrosis. Their eldest son who is now 25 years old is interested in marriage and has requested testing to determine if he is a carrier of a cystic fibrosis allele. He has previously tested negative for the same sweat test which Jeremy received. What is the pre-test risk that Jeremy’s brother is a heterozygous carrier of a cystic fibrosis allele, given that he does not have the disease?

A) 1/10  
B) 1/4  
C) 1/2  
D) 2/3  
E) 3/4

Refernces:
1. For information about the sweat test used to diagnose CF patients, please visit the following website: www.cysticfibrosismedicine.com/htmldocs/CFText/sweat.htm
2. For the disease profile of CF, treatments, support groups and clinical trials, please see the website: www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/cf.shtml
3. The following two references are for cystic fibrosis research, both general information and that related to question #4 above: