NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Stuck on You: Health Complications Associated with Dysregulation of Ion Movement

by

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Introduction

You are starting your fourth week of a five-week rotation with a local pediatrician. As part of your rotation you get to accompany the physician, Dr. LaToya Robbins, on patient appointments and diagnoses.

A worried mother and father have just brought their two-year-old son into the clinic. You quickly grab your note pad and follow Dr. Robbins to the room where the patient and his family are waiting.

In the exam room you find Dr. and Mr. Johannas and their son, Tyler. Dr. Robbins asks the parents to give her a bit of history on their son and to describe why they have brought him in today. Both parents are of northern European descent and Tyler is their first child. Tyler was born in Belgium, delivered via a vaginal birth weighing 7 lbs. 8 oz, and there were no complications with Dr. Johannas' pregnancy or the birth. Tyler was breast fed for six months and then transitioned to soft foods and baby cereal. Tyler and his parents moved back to the United States when Tyler was seven months old after his mother completed her Fulbright scholarship in Belgium. Ever since he was a baby, Tyler has been on the low end of the growth curve, despite supplementing his breast feeding with formula. Lately, Tyler has been wheezing and coughing, and he had a bad case of bronchiolitis a few months ago; it never seemed to totally clear up as he has had several rounds of antibiotics and still has a recurring cough. His cough is described as "wet and junky" by his parents. His parents report that "every time he gets a cold, his cough seems to last weeks to a month." Also, he has not put on weight since his one-year well-child visit. His parents describe him as "continually hungry" and "eating all day long," although despite this, he continues to loose weight and has intermittent diarrhea. The nurse has already taken vital signs (Table 1).

Table 1. Tyler's vital signs.

Measure	*Result*	*Typical Value for Two-Year-Old Child*
Body Temperature (°C)	37.5	36–38
Pulse (beats per min)	90	80–130
Respiration (breaths per min)	38	24–40
Blood pressure (mmHg)	90/44	86–106 / < 75
O_2 saturation (%)	92%	96–99%
Weight (lbs.)	25 (5^{th} percentile for age)	—
Height (inches)	34 (20^{th} percentile for age)	—
Body Mass Index (BMI) kg/m^2	14 (< 5^{th} percentile for age)	—

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Tyler is a thin, pale-appearing toddler. He is active, running around the exam room, and does not appear to be in any distress. He coughs a few times during the visit, and the cough sounds harsh and wet. His ear and nose exam are normal. He has some redness at the back of the throat. His heart exam reveals normal heart sounds and rate, with good pulses. Lung exam reveals bilateral crackles at the posterior bases, with some expiratory wheezing. There are no retractions or nasal flaring. His abdomen is soft, with normal bowel sounds, although his liver feels mildly enlarged and the abdomen looks slightly distended. Musculoskeletal exam is normal. Skin exam is normal.

Dr. Robbins orders blood tests and a throat culture. A blood draw is completed and a small swab is used to get a sample from Tyler's throat (getting sputum from a toddler is difficult and a throat swab is a good first choice test). After a few days the results come back from the lab; results are listed below. Dr. Robbins looks them over and lets out a "hmm" under her breath. She immediately calls the parents to tell them she would like to order a pair of sweat tests for their son.

Table 2. Lab results.

Measure	*Result*	*Typical Values*
Serum thyroxine (T_4) (ng/dl)	0.99	0.86 – 1.40
Serum Triiodothyronine (T_3) (pg/dl)	4.06	3.34 – 4.80
Hematocrit (%)	48	29-41
Fasting Plasma Glucose (mg/dl)	100	70-110
Blood Sodium (mmol/L)	129	135-145
Blood Potassium (mmol/L)	3.5	3.4-4.7
Blood calcium (mg/dl)	8.6	8.5-10.2
Plasma pH	7.32	7.40 ± 0.05
pCO_2 (mm Hg)	50	40
pO_2 (mm Hg)	65	90-100

Table 3. Throat culture.

Bacterium Species	*Result*
Staphylococcus aureus	Positive
Haemophilus influenzae	Negative
Pseudomonas aeruginosa	Positive

Questions

1. What signs and symptoms does Tyler have?

2. Are any of the vital signs or lab results abnormal? If so, which ones and in which direction?

3. What is a sweat test?

4. Pilocarpine is used in the sweat test to induce sweating. Based on your knowledge of autonomic control of sweating, what do you think is the mechanism of action for pilocarpine?

5. What is cystic fibrosis? (Helpful information is available on the websites hosted by the Cystic Fibrosis Foundation, National Institutes of Health, and Mayo Clinic.)

**** Once you have answered Questions 1–5, contact your instructor to get the results of Tyler's sweat tests. ****

6. What do you think is a reasonable diagnosis for Tyler? What evidence/symptoms support this diagnosis?

Part I – Genetics and Cell Biology

The Basics

Cystic fibrosis (CF) is caused by genetic factors, but it can also be influenced by environmental factors. People with CF have inherited two of the roughly 2,000 possible mutations of a gene on chromosome 7. This gene normally codes for a gated Cl^- channel (CFTR) in the plasma membranes of cells. The severity of the disorder depends on which specific mutations one has inherited. It also depends on the conditions of several other proteins required for proper CFTR function. Environmental factors can also impact severity (especially for respiratory infections), but the details of how all environmental contributors impact this disease have not been systematically investigated.

1. If a person inherits only one of these mutated genes, s/he will *not* develop CF. Consequently, CF is best characterized as (circle one):

   (a) autosomal dominant.

   (b) autosomal recessive.

   (c) X-linked dominant.

   (d) X-linked recessive.

2. Sketch out a simple diagram for the formation of a eukaryotic cell membrane protein. Make sure to list (a) transcription, (b) translation, (c) post-translation modification, (d) folding, and (e) incorporation into the membrane. *Briefly* explain each step.

Mutations

Mutations to the *CFTR* gene are classified according to the clinical severity of the mutation. As one progresses down the list from Class I to Class VI, the symptoms generally become less severe.

Table I.1. Classes of *CFTR* gene mutations for cystic fibrosis.

Class	*Effect of Mutation*
I	Early stop codon, truncated CFTR
II	CFTR mis-folding, mis-targeting, instability
III	Dysfunctional gating of CFTR
IV	Ineffective Cl^- conductivity by CFTR
V	Defective splicing of *CFTR* mRNA
VI	Reduced CFTR longevity in membranes

3. Using the Roman numerals in Table I.1 above as symbols for genes, come up with three combinations of two different genotypes:

   (a) least severe phenotype: _____

   (b) most severe phenotype: _____

   (c) intermediate phenotype: _____

Most of the 2000 known mutations to the *CFTR* gene are Class I mutations, yet only 5–8% of people with CF have a Class I mutation. Instead, about 90% of people with CF have at least one F508del mutation, which is classified as a Class II mutation. The F508del mutation is a deletion of the codon for phenylalanine at position 508 of the protein. This deletion alters the protein folding and means the protein does not get trafficked to the cell membrane appropriately, decreasing CFTR function and therefore chloride ion secretion. Thus, one might expect those homozygous for F508del to have especially severe cases of CF, and some do, but there is wide variation in the severity of such cases. For example, siblings each with two copies of the F508del can have very different clinical severities.

4. How can the variability of CF severity seen among those who are homozygous for F508del be explained?

Treatments

To date, many drugs to treat CF have become available. The drugs are targeted to treat different classes of mutations (see Table 2).

Table I.2. Drugs for cystic fibrosis

Class of Mutation	*Drug Mechanism of Action (and Examples)*
I	Read-through agents (gentamycin, tobramycin)
II	CFTR correctors (ivacaftor, tezacaftor)
III	CFTR potentiators (lumacaftor)

Read-through agents cause ribosomes to ignore early stop codons. CFTR correctors permit improved passage of CFTR to the plasma membrane by several mechanisms, which include causing CFTR to fold properly (by molecular chaperones), and thus to escape early destruction. CFTR potentiators improve the capability of the CFTR channel to open, thereby improving Cl^- conductivity.

Clearly, the available treatments are not equally effective against all types of CF. Modern treatment of CF requires identifying which mutations an individual with CF possesses, and then choosing the most-appropriate drug(s). This is an example of "personalized medicine" or pharmacogenomics (how genotype impacts response a drug).

5. What drug(s) do you think would be the best treatment for the 90% of CF individuals who have at least one F508del mutation?

6. What drug(s) do you think would be the best treatment for CF individuals who have one F508del mutation, and one Class I mutation?

7. In October of 2019, the Food and Drug Administration (FDA) approved the use of Trikafta for use in CF patients. Trikafta is a blend of three drugs: elexacaftor, ivacaftor, and tezacaftor. Who (as in which type of *CFTR* mutation genotype) do you think this therapy would be best suited to help?

8. What percentage of CF patients may possibly benefit from Trikafta®? (See the October 21, 2019, FDA press release "FDA approves new breakthrough therapy for cystic fibrosis" at <https://www.fda.gov/news-events/press-announcements/fda-approves-new-breakthrough-therapy-cystic-fibrosis>.)

Gene Editing

Although CF can be treated, it cannot be cured as of yet. Gene therapy could potentially cure CF by targeting the cause of the disease, the mutant gene, by replacing it with a healthy gene. In the past, viral vectors have been used to introduce healthy *CFTR* genes into patients, but without consistent success. A new technique called the CRISPR/Cas9 system holds significant promise as a means of introducing typical genes in the place of mutant ones. Once the CRISPR/Cas9 system can be modified to specifically target the *CFTR* gene, years of clinical trials lie ahead before the treatment would become routine.

Watch the following video that describes CRISPR/Cas9:

- *Osmosis.org*. 2018. CRISPR-Cas9 and the Age of Gene-Edited Humans. Running time: 5:13 min. <https://youtu.be/1viRt8jV-vk>

9. Briefly explain what CRISPR/Cas 9 is and describe how it could help treat genetic disorders.

10. If a researcher wanted to attempt this procedure to cure CF, which chromosome will CRISPR/Cas9 target?

Part II – Cell Physiology

Water and Ions

Diffusion of solutes (and of water via osmosis), is a process that is important for several aspects of human physiology. In order to understand how mutations in a chloride channel can affect health, it is important to first understand diffusion, osmosis, and osmolarity.

Use Figure 1 to answer the following questions.

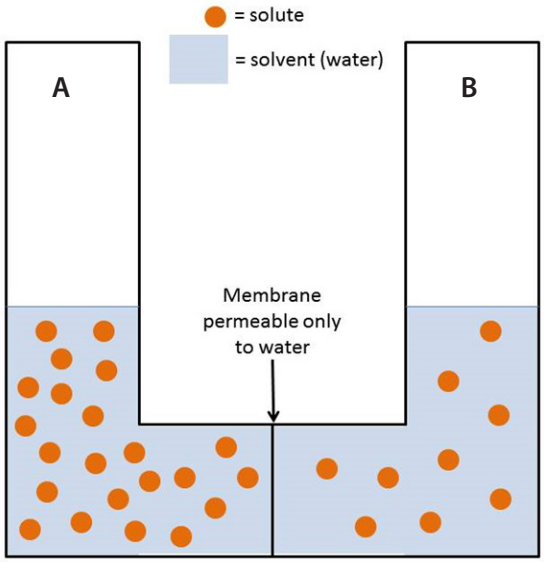

Figure II.1. Solution divided by a membrane only permeable to water.

1. How many solute molecules are in the solution on side A? On side B?

2. Define *osmolarity*.

3. Assuming side A and side B both contain 1 L of water and that the solutes are osmotically active, what is the osmolarity of each side? Make sure to include units.

4. Which side has the higher osmolarity?

5. Draw what you expect to happen to water movement across the membrane in the center of the tubes. Assume that the solutions were just added to side A and side B. Explain the rationale for your answer.

Assume we change the membrane located between the tubes as in Figure 2. It is now permeable to water and solutes.

6. What is the osmolarity of Side A? Of Side B?

7. Using Figure II.2, is Side A hypertonic, hypotonic, or isotonic to Side B?

8. In Figure II.2 as drawn, do you expect *net* movement of water? Why or why not?

9. Now, assume that the membrane contains an active transport pump that moves solute from Side A to Side B. This pump works at a rate that can overcome the rate of solute diffusion. What is going to happen to the movement of water? Why?

10. In a human cell, how does water cross a cell membrane?

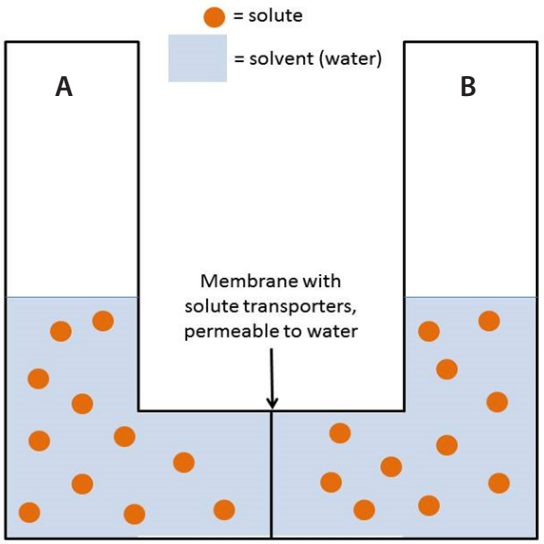

Figure II.2. Solution divided by a membrane permeable to water and the solute.

Cellular Impacts of Cystic Fibrosis

11. What do the letters CFTR, in the CFTR channel name, stand for?

This channel is located on the apical side of epithelial cells and is a part of a group of channels called ATP-binding cassette (ABC) transporters. In people with cystic fibrosis, this channel does not function properly due to one of several genetic mutations. Being homozygous for mutations in the *CFTR* gene results in pronounced changes in the epithelial cells of the lungs, digestive tract, reproductive tract, and sweat glands. The illustrations below are somewhat simplified but represent the CFTR channel's role in the lung (Figure 3) and in the sweat glands (Figure 4). Use these figures to help you answer the next set of questions. Note that movement of chloride ions is typically followed by movement of sodium ions and that water follows sodium.

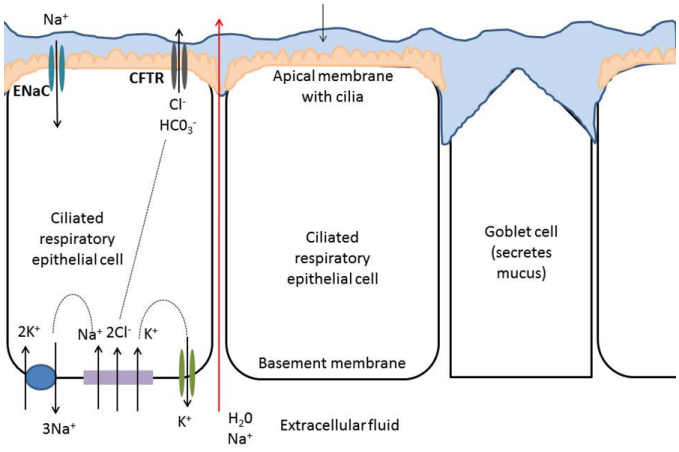

Figure II.3. Schematic of the epithelial cells of the respiratory tract and the role of CFTR in the lungs. The epithelial sodium channel (ENaC) is also shown. These two channels both play a role in keeping the surface moist and work in a balance of anion (Cl^-) secretion and Na^+ reabsorption. If the CFTR is not functional, the ENaC tend to become overactive, thus upsetting the balance. The airway surface liquid (ASL) helps to coat the apical surface of the cells and cilia. Mucus is produced and sits along with surface of the ASL to create a mucus gel layer.

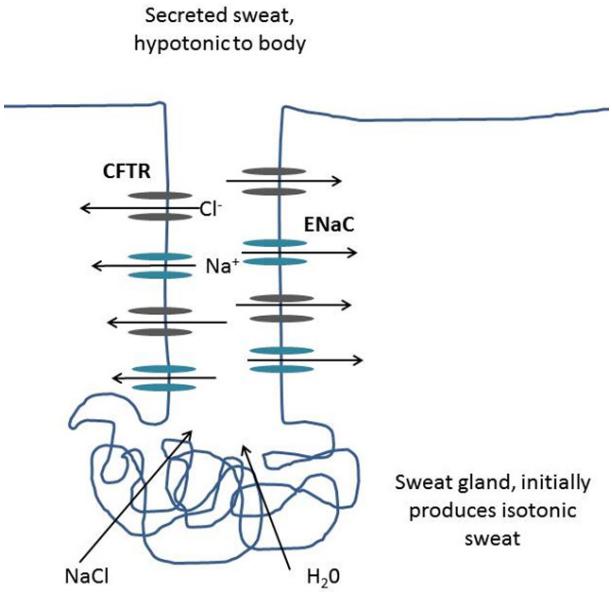

Figure II.4. Schematic of the role of CFTR and the ENaC in the sweat glands. Note the direction here; both chloride and sodium ions are reabsorbed by the body. Chloride moves across the membrane and its flow helps draw sodium across. Again, these channels work in a balance to maintain the NaCl concentration of sweat.

12. Normally, the airway surface liquid of the respiratory tract is a watery saline solution. Several ions and channels are involved in maintaining this solution. Using Figure II.3, describe how cystic fibrosis could lead to thick, sticky mucus in the airways.

13. Explain why individuals with cystic fibrosis have salty sweat.

Part III – Respiratory Function

Respiratory Function and Predictions

Cystic fibrosis can cause problems for several physiological systems, including the respiratory system. People with CF produce thick, sticky mucus in their airways instead of watery saline.

1. List the path that air takes, going from nose to alveoli.

2. What is the function of the mucus in the airways?

3. What is the function of the mucociliary escalator? What are its two major cellular components?

4. What effect do you think the sticky mucus will have on someone with CF? Explain.

5. Poiseuille's law states that resistance to flow is affected by length of the system, viscosity of the substance (here, air) flowing through the system, and the radius of the tubes in the system. In terms of the respiratory system, which of the above is typically going to have the biggest impact on resistance to air flow? Why?

6. What do you predict the mucus and inflammation from cystic fibrosis does to airway resistance? To vital capacity (the greatest volume of air that can be expelled after a deep breath)? To alveolar ventilation (volume of air entering and leaving the alveoli per minute)?

7. Knowing what you do about respiration, what do you think cystic fibrosis does to oxygen carrying capacity and pO_2 levels in the blood? Explain.

Tyler's Results

Dr. Robbins discusses cystic fibrosis with Tyler's parents. She tells them that with cystic fibrosis, the sticky mucus lining the airways leads to a defective cilia/mucus escalator, chronic infection and inflammation, and decreased ability to breathe efficiently. The mucus builds up and restricts the inflamed airways (bronchi and bronchioles) making it more difficult for the person to draw in air.

Dr. Robbins refers Tyler to the nearby, large pediatric hospital for further workup including a lung function test. Results from this test are shown in Table III.1.

8. Are any of Tyler's lung function results abnormal? If so, which ones and in which direction?

Table III.1. Lung function test.

Measure	*Result*	*Normal Range*
Breaths per min	38	20–30
Tidal volume V_T (ml)	90	100–130
Anatomic dead space volume V_D = 33 ml		

Use the equations below for Questions 9 and 10.

$$V_E = f \times V_T \qquad V_A = f \times (V_T - V_D)$$

V_E = respiratory minute volume; f = breaths per minute; V_T = tidal ventilation; V_A = alveolar ventilation; V_D = anatomic dead space.

9. What are Tyler's respiratory minute volume and alveolar ventilation? (Show your work and include units.)

10. Calculate the respiratory minute volume and alveolar ventilation for a child who is healthy (use values V_T = 120, f = 30). How do Tyler's values compare to a healthy child's?

11. What do the above results tell you about Tyler's breathing and the amount of air reaching the exchange surfaces (alveoli)?

12. Hypoventilation decreases the partial pressure of oxygen in the alveoli. The normal pO_2 in the alveoli is 100 mm Hg. Use the graph below (Figure III.1) to fill out the table below (Table III.2) to determine how changing alveolar ventilation can affect oxygen carrying capacity.

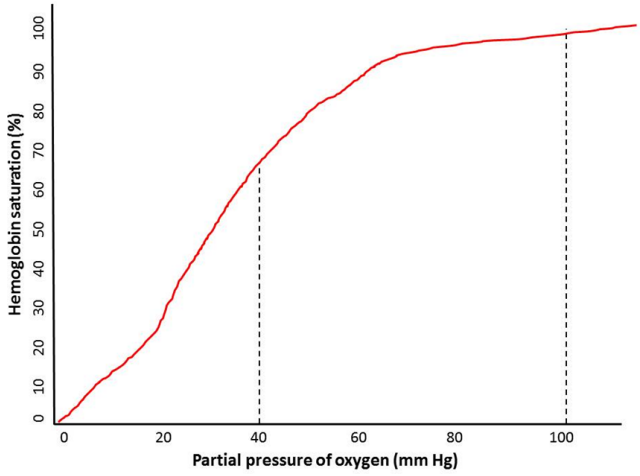

Figure III.1. Impact of changing alveolar ventilation on oxygen carrying capacity

Table III.2. Impact of alveolar ventilation on oxygen carrying capacity.

Alveolar pO_2 (mm Hg)	*Saturation of Hemoglobin (%)*
100	
80	
60	
40	
20	

13. Above, in Question 7, you predicted what cystic fibrosis would do to oxygen carrying capacity and pO_2 levels in the blood. Compare your prediction to the data from above. Were your previous predictions correct? If not, what adjustments can you make to your answer now that you have more information?

Part IV – Reproductive System

People who have cystic fibrosis can potentially have reproductive deficits, which are generally more serious in mature males than in mature females.

CF and Female Reproductive Physiology

We know that people with CF produce highly viscous mucus, and we know that mucus plays important roles in the female reproductive system. Hormonal changes that occur during the menstrual cycle alter the composition and properties of that mucus, so mucus from different stages of the cycle differs. Additionally, the cervix can produce mucus that differs in composition from that produced by the uterine lining (endometrium).

1. Under the influence of estrogens (EST), what are the characteristics (e.g., viscosity, thickness, composition) of mucus produced by the cervix? Normally, how does this mucus influence fertility?

2. Under the influence of progesterone (PRG), what are the characteristics of mucus produced by the cervix? Normally, how does this mucus influence fertility?

3. Under the influence of PRG, what are the characteristics of mucus produced by the endometrial lining? Normally, how does this mucus influence fertility?

4. Which one of these three types of mucus (cervical under EST, cervical under PRG, or endometrial under PRG), if made thicker by the *CFTR* mutations, should have the *least* effect on female fertility, and why?

There are treatments that can overcome these difficulties, and women with CF can become pregnant. The effect of CF on body weight, and especially on body fat, can also have reproductive consequences.

5. Briefly sketch or list the steps in the production of progesterone and estrogen. Start from cholesterol.

6. If estrogens are synthesized directly from testosterone, then which enzyme is responsible for the conversion of testosterone to estrogen and where is this enzyme found in the body?

7. Define puberty.

8. While the exact drivers of puberty are not known it is clear that many hormones are involved. Additionally, there are several environmental and physiological factors that can play a role. Puberty is often delayed by a year or two in females with CF, especially those with lower-than-average body weight. What do you think might partially causes this delay?

Amenorrhea is the absence of menstrual cycles for a period of six months or longer. Not uncommonly, amenorrhea is experienced by mature females who are distance runners, or who have anorexia nervosa, or who have CF, or who are starving.

9. What explains why all of these women may experience amenorrhea? What treatment(s) might be used to solve this problem?

CF and Male Reproductive Physiology

Most men with CF are infertile. This infertility is usually not due to a problem with spermatogenesis. Normal numbers of immature sperm cells are present within the testes of men with CF.

Study the table below of values from males with CF. Percentages are comparisons to typically functioning males.

Table IV.1. Sperm cell counts.

Site of Sampling	*Percentage of Viable* Sperm*	*Percentage of Active† Sperm*	*pH*
Testes	99%	rare	7.3
Epididymis	95%	rare	7.3
Urethra	0–2%	0–1%	5.1
Ejaculate	0–2%	0–1%	5.0
*Viable means the sperm are alive. †Active means the flagella are moving.			

10. In which sampling site(s) do values appear to be close to normal? Appear to be anomalous?

The problem may be localized to the portion(s) of the male reproductive system that is/are located between the epididymis and the urethra.

11. Which tube of the male reproductive system connects the epididymis to the urethra?

12. Which accessory glands of the male reproductive system are located between the epididymis and the urethra?

13. Research the condition of the tube in Question 9 above in men with CF. (The Cystic Fibrosis Foundation has helpful information.)

14. Research the condition of the seminal vesicles of men with CF.

15. How do your answer to Question 12 above and the data from the table above explain the inactive condition of the few viable, active sperm found in the ejaculate of some men with CF?

16. If the male has intact epididymides, then viable, mature sperm may be removed from them by biopsy. When one mixes these viable, mature sperm with viable eggs, fertilization rarely occurs. Remembering that sperm cells are not the same as semen, propose an explanation for this lack of achieving fertilization.

Such sperm have been treated with modified assistive reproductive technology (ART), including a complicated sequence of exposures to various chemicals required for final maturation and capacitation of sperm cells. However, when such sperm are mixed with fertile eggs, the production of a zygote is, once again, rare.

17. Can you think of a relatively simple and more reliable way of producing a viable zygote? If not, research one, and report on it.

Part V – Microbiology

Cystic fibrosis patients often experience recurrent or chronic respiratory tract infections. The thick sticky mucus present in the lungs means lung infections are more common and take longer to clear due to the impaired mucociliary clearance. The presence of different organisms has a significant impact on the long-term outcomes for patients with CF as chronic infection and inflammation can lead to scarring and decreased performance of lung tissue, which decreases overall pulmonary function. As part of a normal care plan, CF patients have throat swabs or sputum culture tests performed every three months. This helps clinicians detect and identify bacterial or fungal organisms that might be present and allows for proper antibiotic administration.

A respiratory therapist requests that the patient do a huff cough to see if any sputum can be brought up. If the cough is productive, meaning sputum is produced, the sputum is collected and taken to the lab to be cultured. If the cough is non-productive then a swab is taken at the back of the throat and the resulting swab sample is cultured. The following results are available 24–72 hours after the sample is taken in the case of most organisms, but results can take several weeks for slower growing species.

Results of CF culture from sputum bronchus in 2.5 year old male:

- 2+ organisms are compatible with mixed respiratory flora
- Rare: *Staphylococcus aureus*; methicillin sensitive; 1+becta lactamase POS
- Heavy: *Haemophilus influenzae* beta lactamase neg
- Moderate: *Escherichia coli*
- Rare: *Moraxella catarrhalis*

1. Based on the culture results what do you think "rare," "heavy," and "moderate" mean? Which organism listed had the most growth in culture?

2. Explain why no viruses are listed in the results.

3. All bacteria listed from the culture results are part of the common respiratory flora except one. Identify the non-respiratory organism and speculate on why a non-respiratory organism is present in a sputum sample.

Gram staining is a technique used to help identify which antibiotics may be appropriate for treating bacterial infections. Gram staining differentiates bacteria into two large groups based on constituents in the cells wall. A series of reagents are added to bacterial cells that interact with the cell wall components resulting in either a dark purple or a light red/orange color. Dark purple cells are Gram positive while the lighter red/orange are Gram negative.

Table V.1. Gram staining and morphology of sample bacteria.

Organism	*Gram Stain and Morphology*
Staphylococcus aureus	Gram positive cocci in clusters
Haemophilus influenzae	Gram negative coccobacilli
Escherichia coli	Gram negative bacilli single cell
Moraxella catarrhalis	Gram negative diplococcus

4. Based on the organism cultured and the gram stain description given in Table V.1, draw examples of what these four organisms would look like below. If it is "purple" please shade in the organism and if it is "red/orange" do not shade it in. Label your organisms below each drawing.

The patient has had a prolonged cough and confirmed bacteria growing from the sputum culture. Antibiotics are prescribed. The doctor is considering three options: amoxicillin, bactrim (TMP-SMX), or vancomycin.

5. Look up the mechanism of action for each of these antibiotics.

6. Using the results of the tests of the patient's sputum sample, choose which of the antibiotics would be the best choice to use for treatment in this case and explain why.

In patients with CF, mitigating antibiotic resistance is highly important. These patients often have recurrent infections with the same organism and development of antibiotic resistance can limit the antibiotic arsenal that can be used. As a result, narrow spectrum antibiotics are preferred to specifically target the organism causing the infection. Bacterial resistance can develop as a result of a number of factors and is not just an issue facing CF patients, but is a growing worldwide problem. Development of new antibiotics is rare and the drug discovery process is slow. Currently used antibiotics work by targeting something in the bacterial cell without hurting or minimally hurting the eukaryotic cells.

7. List the five basic methods of action for currently available antibiotics.

Beta lactamases are bacterial enzymes that provide resistance to beta lactam antibiotics. Beta lactam antibiotics have a core structure with a four-atom beta-lactam ring. This core ring mimics the substrate that is used in cell wall synthesis. Four major categories of antibiotics have beta-lactam core structures: penicillins, cephalosporins, monobactams, and carbapenems.

8. Describe three ways (there are many more) that a bacterial cell might be able to protect itself from an antibiotic.

9. Imagine you are working at a pharmaceutical company whose directors have decided to pursue development of a new type of antibiotics. Brainstorm with your group and come up with a new way to target bacterial cells. Be specific and explain your responses: For example, if you are targeting a gram negative cell, then specify that; or, if you are targeting a bacterial metabolic pathway, then describe where and how, etc. Finally give your new invention a drug name.