1. Explain the differences between primary cell culture and the use of continuous cells lines. What are the advantages and disadvantages of using cultured cells as an experimental system?

**Ans:** Primary cultures are derived directly from the animal and will only divide and grow for a discrete time period. Continuous cell lines, in contrast, are derived from transformed tissues (tumors) or embryonic tissues that retain their immortalized character and can be propagated indefinitely. Advantages are experimental ease of manipulation - you can add drugs to the culture medium, microinject the cells, and watch them using microscopy. All of these manipulations are technically more difficult with whole organisms. The drawback of their use is that cells in a dish are in a very artificial state and they lack the normal information that they would otherwise be receiving from other cells and extracellular matrix components in a tissue.

2. One of the central differences between eukaryotes and prokaryotes is that the latter lack internal membranous organelles. Why do eukaryotic cells have these membrane-bound compartments?

**Ans:** Membrane-bound organelles are compartmentalized to allow specific biochemical reactions to take place efficiently. For example, the lysosome is an organelle that contains many proteases used to degrade food. Cells package these enzymes into this organelle so that they don't go around digesting the cell's own proteins in the cytoplasm.

3. You discover a cell that is unicellular, approximately 10µm (microns) in diameter, and divides by mitosis. Is this a eukaryote, a prokaryote, or is it impossible to know based on this information?

**Ans:** It’s a eukaryote because prokaryotes do not undergo mitosis.

4. Which experimental organisms would be best suited for studies on the following topics?
   (a) Development of the vertebrate nervous system (**mice, zebrfish**)
   (b) The physiology of crop plants (**arabadopsis**)
   (c) The cellular targets for a novel antibiotic (**bacteria**)


5. Both light and electron microscopy are commonly used to visualize cells, cell structures, and the location of specific molecules. Explain why a scientist would choose one or the other microscopy technique for use in research.

**Ans:** Electron microscopy is used to visualize ultrastructure of cells down to a few nm. Light microscopy is used to understand cellular organization to ~1200 nm resolution. If you are interested in the organization of the entire cell, for example, it would be difficult to see the entire thing in a cell that had been processed for EM and cut into very thin sections (70 nm). Also, if you wish to conduct an experiment with living cells, then light microscopy is your only choice.

6. Why are chemical stains required for visualizing cells and tissues with the basic light microscope? What advantage does fluorescent microscopy provide in comparison to the chemical dyes used to stain specimens for light microscopy? What advantage does confocal microscopy provide in comparison to conventional fluorescence microscopy?

**Ans:** Because most cells and tissues are transparent, one must stain them with dyes and stains that bind to the sample in order to impart contrast. Fluorescence microscopy is great because 1) the contrast is very high since you are looking at a fluorescent dye against a black background, 2) the fluorescent probes you use can be very targeted to very specific components of the cell (i.e. antibodies to a particular protein). Confocal microscopy allows one to view optical sections of thick specimens, thus removing out-of-focus light that makes the images hazy. Sets of optical sections throughout the thickness of the specimen can be used to build 3-D reconstructions of samples (but I don't think I talked about this in class).
Biology 52: Problem Set for Lectures 3 and 4

1. The gecko is a reptile that has the amazing ability to climb smooth surfaces - even glass. Recent discoveries indicate that geckos stick to smooth surfaces via van der Waals interactions between septae on their feet and the smooth surface.

(a) How is this method of stickiness advantageous to the gecko over covalent interactions?

**Ans:** A covalent interaction would glue the gecko to the surface irreversibly.

(b) Given that van der Waals forces are among the weakest molecular interactions, how can the gecko's feet stick so effectively?

**Ans:** As the interactions are weak, a large portion of the surface area of the gecko's feet must be dedicated to forming these interactions.

When not selling car insurance, geckos climb smooth surfaces.

2. Below is a standard table of the amino acids, and the sequences of a short polypeptide that has one transmembrane domain.

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MKRENSDNHIALVVLAIIEGGLTAFFGFEDNIKELDNK

Underline the predicted transmembrane domain in the polypeptide above.
3. In what kinds of microscopes would you find a dichroic mirror?

Ans: In a fluorescence microscope.

4. Explain two ways that we know many membrane proteins can diffuse laterally in the plasma membrane in cells.

Ans: Fluorescence recovery after photobleaching (FRAP) of fluorescently-labeled membrane proteins and single-molecule imaging of GFP-tagged membrane proteins. We discussed both of these experiments in lecture.

5. You are a biochemist studying the properties of cell membranes across species of birds from around the world. The labels come off of two tubes: the first is a penguin sample (from Antarctica) and the second is from a macaw (a parrot that lives in tropical South America). As a part of your analysis, you quantitate the amount of cholesterol, saturated, and unsaturated fatty acids present in both of the membrane samples. How would you use this information to identify which samples came from which bird and why?

Ans: Decreased unsaturated lipid and decreased cholesterol lead to more fluid membranes. Also, membranes are less fluid at colder temperatures. Thus, the animals that live in colder habitats would require less of these molecules in their membranes to maintain proper fluidity. However, the presence of cholesterol also prevents membranes from freezing. Thus, the best metric would be increase unsaturated lipids.

6. An enzyme that adds phosphate to a protein is called a _____kinase_____. An enzyme that removes phosphate from a protein is called a _phosphatase__.