1. Two cultures of *E. coli*, each containing \(10^8\) cells/ml, were grown in enriched media. One culture was aerated, the other was not. It was found that, while both cultures increased from \(10^8\) to \(10^{10}\) cell/ml, the unaerated culture used 19X more glucose than the aerated culture. Why is this so?

The unaerated culture had the same energy needs as the aerated culture in order to produce the same amount of growth, but the unaerated culture could only gain energy (ATP) from glycolysis, which is 19X less efficient than aerobic respiration. Thus the unaerated culture had to break down 19X more glucose to get the same amount of energy as the aerated cell could get from one molecule.

\(C^{14}\) labeled glucose was added to the cells in each flask. In what product(s) is the radioactive carbon found in each culture?

<table>
<thead>
<tr>
<th>aerated</th>
<th>unaerated</th>
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<tr>
<td>(CO_2)</td>
<td>(CO_2 + ) ethanol or lactic acid</td>
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2. A bacterium having a very bad day suddenly finds itself on the floor of a chemical plant where it encounters a variety of compounds that affect its respiratory metabolism. First it falls into a pool of cyanide, then it rolls into a pool of dinitrophenol and finally comes to rest in a greasy spot of fatty acids. Explain how each of these encounters would affect its rate of O₂ utilization, ATP synthesis and glucose breakdown. Assume full recovery from each encounter before the next encounter occurs.

**Cyanide** - cyanide will inhibit the electron transport chain, preventing electrons from reaching O₂ and preventing protons from being pumped out, thus preventing ATP synthesis. If electrons can't be disposed of, then NADH will not be reoxidized and glycolysis will initially be inhibited, unless it can carry out fermentation to meet its energy needs. Then glucose breakdown will increase to supply those needs with a less efficient pathway.

**Dinitrophenol** - DNP uncouples electron transport from oxidative phosphorylation by making the mitochondrial membrane leaky to protons, causing O₂ use to increase while ATP synthesis ceases. This will increase the rate of glucose breakdown since NADH and FADH₂ can be oxidized more quickly making NAD⁺ more readily available, thus stimulating glycolysis and the TCA cycle.

**Fatty acids** - will form acetyl CoA which enters the TCA cycle. Both fatty acids and acetyl CoA as well as citrate are allosteric inhibitors of glycolytic enzymes slowing their activity and thus the rate of breakdown of glucose. O₂ utilization and ATP synthesis may stay the same or increase depending on how fast the fatty acids are metabolized.
3. The synthesis of ATP as a result of the electron transport system is dependent on the delivery of electrons and hydrogen ions by NADH and FADH$_2$ to the inner membrane of the mitochondria in eukaryotic cells or the cell membrane of prokaryotic cells. For each of the situations below, give the maximum number of ATP generated.

   a. one molecule of FADH$_2$ produced during the TCA cycle - 2

   b. one molecule of NADH produced during the TCA cycle - 3

   c. one molecule of NADH produced by glycolysis in a prokaryotic cell - 3
4.

In the diagram of a mitochondrion shown above indicate with a labeled arrow the location of the following:

A) stroma   C) TCA cycle   E) ATP synthase
B) cristae   D) electron transport chain

Explain **how** and **why** the following will affect the rate of ATP synthesis and the rate of $O_2$ utilization in a liver cell:

a) pyruvate kinase activity is inhibited -

This would effectively stop glycolysis at the final reaction and prevent pyruvate from being formed and utilized in the TCA cycle. No NADH would be made and no electrons from them would be available to travel down the electron transport chain to use $O_2$. Likewise no ATP would be synthesized since no proton gradient would be built up.

b) ADP becomes unavailable -

This would halt ATP synthesis at the ATP synthases since they require ADP and Pi to make ATP. This would, in turn, halt electron transport since the two processes are tightly coupled by the proton gradient which would be increasing due to the continued electron flow. Finally the gradient will get
so steep that electrons can no longer move down the chain to \( O_2 \). Thus \( O_2 \) utilization would decrease as well.

c) oligomycin is added to the cell -

This would halt ATP synthesis since the molecule blocks the proton channel in the ATP synthase. This would also halt electron transport and thus \( O_2 \) utilization because these two processes are tightly coupled. The \( H^+ \) gradient would become too high to permit electrons to travel down the electron transport chain thus preventing \( O_2 \) utilization.