DEMOS of the
BIOCHEMISTRY of PHOTOSYNTHESIS, RESPIRATION, & FERMENTATION
and their LIKELY EVOLUTION
Making Bioenergetics EXCITING!

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RATIONALE: The major biochemical processes of life are well understood, and often play roles in the expression of various genetic disorders (to be introduced in the genetics unit). Respiration, in particular, can help us to understand how our body handles increased food intake & less exercise vs decreased food intake & more exercise. Unfortunately, bioenergetic processes appear rather complex, and are typically shown with unfamiliar chemical names and/or symbols, all very abstract. However, we can achieve some level of understanding and insight to these processes by presenting the essential components in a dynamic and graphic way that emphasizes the key parts (especially those with familiar names), and conveys the essence of biological efficiency in the conservative processes of repeated **cyclic and coupled reactions**. By doing this, students learn that seemingly complex materials and processes are really **not that complex**, and reveal **patterns** that they can understand. This lays the **conceptual foundation** upon which they can build a more detailed knowledge that they may encounter in college level courses. In addition, an overview of certain molecular processes can show how they probably **evolved**. For example, the relatively inefficient energy extraction steps of anaerobic fermentation can be seen in the glycolysis part of cellular respiration, where greater efficiency is accomplished when oxygen functions as the universal hydrogen acceptor (producing water as the waste product).

PHOTOSYNTHESIS: This Demo is intended to dramatize the **essential function of chlorophyll** in photosynthesis, namely to **absorb light energy**, and use that energy to **split off hydrogen** from water, releasing oxygen as a "waste" product. This provides energized hydrogen for the **dark phase**, where it's combined with CO_2 and thereby transforms that (kinetic) solar light energy into the (potential) chemical bond energy of carbohydrates). See the **attached diagram and key**.

In order to dramatize this, I dress up as a chlorophyll molecule, all in green! (I had a green smock made, with the molecular structure of chlorophyll imprinted in big letters on the front.) Thus attired, I hold a handful of wrapped candies ("energized H") in one hand, then move into the beam of strong light coming from the overhead projector, whereupon I start jumping around, making excited sounds, and tossing the "**energized hydrogen atoms**" (candies) out into the class. I've also been known to touch a bucket of water while "energized", and pulled a pressure bottle of oxygen out of the bucket, while tossing the "hydrogen" candies! Be sure that students realize where the energy comes from (light, NOT oxygen!) Oxygen is a waste product of photosynthesis! And the Sun is the ultimate *source* of the light. If I do a repeat performance, I insist that the class shouts out what's in the bucket before I get excited (water), what's in it afterwards (oxygen), and what I tossed out into the class (energized H). This is especially effective just before lunch, or at the end of the day!

RESPIRATION: This is to dramatize the **essential function of respiration**, namely the stepwise **extraction** of **energy** from the chemical bonds of glucose, and its **concentration** into the high-energy bonds of ATP **("universal energy-transfer molecules")**, which are now available anywhere in the cell as an immediate source of energy for any reaction requiring it. This also vividly demonstrates examples of the many **cyclic processes** typical of **cellular metabolism**, where the same molecules are used over and over again, a form of **molecular efficiency**. I do two things (while showing the attached diagram of **Cellular Respiration**):

1. To demo the **ADP-ATP cycle**, I dress up to look like an ADP molecule (similar to my "chlorophyll" attire, only in red). I have a bottle of pink-tinted water (or pink lemonade) in an old "Liquid Energy" detergent bottle (well rinsed!), inserted in an empty 5# C&H sugar bag. The stage is set. I come in as ADP (tired and listless), looking for some energy, I find the bag of sugar, pull out the bottle, pour a bit of the pink juice into a beaker, take a swig, I'm suddenly energized and pick up a little card or box with PO_4 printed on it, becoming ATP, and I rush around energetically, looking for a student who appears a bit listless, toss my PO_4 card at him/her along with a loud "blehhhh" (release of my "energy" to the person; doesn't he/she look

more energized now?), then go back as ADP, listless and lacking energy, to get another energy fix. I repeat this a few times, emphasizing that this cycle is going on *right now*, in all their cells constantly, just to keep them alive, and maybe, if they listen to their cells in a quiet room, they might just hear the little squeels of ATP molecules giving up their PO_4 and energy just so they can live! I enhance this by wearing a sign on a string around my neck, with ADP on one side, and ATP (in red) on the other, and I flip it over to show whichever form I'm in. I also present overlays on the overhead (or a PowerPoint sequence) which illustrates what is happening chemically when the PO_4 group attaches to ADP, turning it into ATP, and back again to ADP when the PO_4 group is released elsewhere in the cell (with its chemical energy) to an energy-using reaction. Follow this with the Krebs Cycle demo (below).

2. **Glycolysis:** If you have introduced **anaerobic fermentation** earlier (as part of your introduction to bio-energetics, showing fermentation as a relatively simple process by which relatively primitive cells - bacteria and yeasts - extract energy from their cells - see below), you can point to the **Pre**-Kreb's Cycle sequence (glycolysis) as mostly the same sequence that was found in fermentation. This is rather clear evidence that the more efficient process of respiration must have evolved as a modification and extension of fermentation, where oxygen (accumulated from its production by photosynthesis) now serves as the ultimate hydrogen acceptor (forming water), and allows more energy to be extracted and transferred to the formation of ATP. Point out that the respiration of one glucose molecule produces 38 ATP molecules net (55% efficiency) vs 2 ATP molecules net (5% efficiency) from fermentation.

3. For the **Krebs (citric acid) Cycle**, I stand with a circle of 4-5 students around me (enzymes in a mitochondrion), and I'm dressed as a **4C** (4-carbon compound). One student hands me a bottle of vinegar (**2C** Acetic Acid, partial breakdown product of glucose metabolism), making me a **6C** Citric Acid molecule (you can hold up a lemon or orange, if you like, with "**6C**" marked on it). Holding out a sheet of paper with two big **CO_2**s and some **H**s on it, I slowly swing around while the "enzymes" (students surrounding me), one by one, tear off my **CO_2**s or **H**s, causing me to **shout** "ATP" (releasing energy), and reducing me (actually oxidizing me) stepwise back to the original 4C compound, ready to pick up another acetic acid molecule (and sheet of paper) and repeat the cycle. If you really want to get elaborate, you can have students standing by to receive each H atom to combine with two O atoms (supplied by other students) to make water. You could also have a chain of student "enzymes" taking glucose (6C) and breaking it down finally to Acetic acid (2C) that is handed to you. It's fun, it's crazy, and it's memorable. I've had some students who have even put this whole scenario to music, and done the "Respiration Rap", or the "Bioenergetics Ballet"! Suggest this, and you might have takers, maybe for extra credit? Be sure to make a video if they do it, and send me a copy!

WHERE DOES RESPIRATION HAPPEN? Be sure to emphasize that **respiration** occurs in **both plants** and **animals**, as their means of extracting energy from carbohydrates for their biochemical needs of life. You might ask for examples of organisms in whose cells we could expect to find **mitochondria**. Be sure that students include some plants in their answers, not just animals.

PRACTICAL APPLICATIONS and EXTENSIONS of CELLULAR RESPIRATION:
How respiration relates to over-eating, lack of exercise, dieting, working out, cramps, muscle soreness.
After dramatizing respiration, it's helpful to review the process with the **Cellular Respiration** diagram (attached, with key) using the overhead (while students fill in the blanks on their copies). When done with the main process, since the reactions are all **reversible**, you can show (on the diagram) how (if we're not very active, but keep eating) PGA can "overflow," forming glycerin, and pyruvic acid "overflows" to form various fatty acids, then the glycerin + fatty acids can combine to build up our lipid (**fat**) reserves! We **gain weight**! Likewise, excess acetic acid can be modified into various amino acids that can be combined to form **proteins**.

On the other hand, if we **exercise**, creating demand for more energy than is immediately available from the glucose in our blood, our liver breaks down the glycogen stored in our liver. If we go on a sustained **weight**

loss program, exercising **and** reducing food intake, our body breaks into our fat reserves, producing fatty acids and glycerin that enter the respiration process where the needed energy can be extracted. If we are captured, and deprived of nearly all food for an extended time, and our fats get all used up, we start breaking down our muscles (proteins), which enter the respiration process at the acetic acid (or other) level, until either our heart or our brain can no longer function, and we die of starvation.

You can also show, in the diagram, where **fermentation** branches off (from pyruvic acid), producing alcohol and CO_2 in yeasts and some bacteria, or **lactic acid** in our **muscles** (if we haven't provided sufficient oxygen to allow respiration to proceed). Lactic acid causes muscle **cramps** and **muscle soreness**. We can reduce the chances of cramps and soreness (or reverse the process by using up the lactic acid) if we **get warm** (warm up before exercising; massage or use heat on a cramp, or take a hot bath), or take a series of deep inhalations ("**suck wind**" before a heavy physical exertion, or during a cramp). Both of these types of effort super-charges our blood with oxygen and increases the circulation needed for the greater oxygen demand of respiration.

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FERMENTATION: If you want to show how these bioenergetic processes probably evolved (see **The Evolution of Bioenergetics**, below), be sure to demonstrate the key steps of anaerobic fermentation. This should be accompanied with labs in which students examine with their microscopes living yeast cells that have been in grape juice overnight vs yeast cells recently placed in tap water (same mass of yeast cells per unit of liquid volume). Prompt students to notice the relative sizes of cells, the number of budding cells, the relative number of cells, and the relative presence of bubbles or air spaces (CO2). You should also set up three thermos bottles as a demonstration, each with a thermometer CAREFULLY inserted (using glycerine) through a two-hole stopper into the contained liquid, and an exit tube with tubing inserted into a beaker of water. Thermos A has grape juice, thermos B has a yeast suspension, and thermos C has a mixture of grape juice and yeast. Each thermos has the same volume of fluid contents, and B & C have the same amount of yeast. Set these up at the end of the day, so students can observe the status and activity of each during the following day. Record the temperatures at start up and every hour during the next day. Temperature in C will rise significantly, while others will remain relatively stable (possibly rising a bit reflecting ambient temperature changes). Bubbles will be seen in beaker C with exit tube coming from thermos C, but not the other thermos's. Testing beaker C's water with pH paper or brom thymol blue will show greater acidity than in other two beakers (due to acidification by CO2 bubbling into it.)

All of these demonstrations will set the stage for discussion of the primary function of fermentation to the yeast cells (extracting energy for their growth and reproduction/budding), with lots of the extracted energy being lost as heat. The CO2 waste product is demonstrated, and you can also demonstrate the abundant residual energy still in the alcohol waste product ("yeast pee") by igniting some alcohol in a Petri dish.

Following these experiences, you can walk your students through the **Fermentation Diagram**, showing a few of the key steps. Show glucose (grape sugar) entering the cell and passing through a series of preliminary steps (in the "clouded" area - where some ATP activation energy is added with phosphate groups, and the 6C glucose is split into two 3C molecules of PGA – PhosphoGlycerAldehyde). Each PGA molecule reacts in a coupled reaction with ADP where the phosphate group and its high energy bond is transferred to the formation of an ATP molecule, and a pyruvic acid molecule is produced. In the following steps, pyruvic acid loses a CO2 gas molecule, and the remainder picks up an energized H that had been removed in an earlier stage (transferred by NAD+), producing the 2C molecule of ethyl alcohol. To emphasize that the alcohol is actually a toxic waste product of fermentation, call it "yeast pee" or "yeast wee-wee"! Also, be sure to demo the fair amount of energy that's still in the alcohol by burning some alcohol in a Petri dish. You should have demonstrated the energy in glucose in your earlier introduction of the carbohydrates by using a Bunsen burner to set fire to a pile of sugar in a Pyrex Petri dish in a darkened room.

If you do the Fermentation presentation, it should precede the study of Photosynthesis (which produces oxygen), then present Respiration (which requires oxygen). This recapitulates the likely sequence for how these processes evolved (see **The Evolution of Bioenergetics**, below)

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PHOTOSYNTHESIS and RESPIRATION: A LAB DEMO2

This demonstration comes after we have worked with both photosynthesis and respiration. It serves as a nice review of some basic concepts, and brings an ecological perspective to bioenergetics.

The demo I use is essentially to set up 2 series of small wide-mouth jars (3 jars/series) with tightly fitted lids (could use large test tubes with rubber stoppers). I prepare enough BTB (brom thymol blue) solution (diluted so it's fairly light, but clearly blue) to fill all 6 jars. Half of this I bubble lots of CO_2 into it (from a CO_2 gas bottle, or add soda water), so it turns yellow. The other half, be sure that it's clearly blue (no CO_2 added). Fill Jars 1-3 with the carbonated BTB, and jars 4-6 with non-carbonated BTB. Place a large healthy *Elodea* sprig or two into jars 1, 3, and 4. Place a water snail or two into jar #6. Close each jar tightly.

DEMONSTRATE what happens when you bubble pure CO_2 into blue BTB (or add soda water), and when you bubble your breath into blue CO_2 (turns yellow in both cases). You may want to explain that BTB merely detects the increased **acidity** of the water when CO_2 is added (H_2CO_3), not the CO_2 specifically, but it *is* a convenient, relatively non-toxic indicator.

Show class the set up before putting them in their respective environments, and insist that they **predict** the outcomes (use the form attached; you may want to collect and check their predictions). Place jars 1-3 in bright light, and jars 4-6 in a **dark** cabinet (no light). Leave them at least overnight, possibly a few days. When you view the jars later, note whether they match the predictions, and discuss any differences.

The prediction requirement and subsequent discussion makes this demonstration an investigative and student-centered one, rather than a strictly "Teacher-Centered Demo."

Sometimes, the snail(s) die. This is sad, but shouldn't significantly change the outcome. Ask students why this is (keeping in mind what bacteria do...).

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THE EVOLUTION OF BIOENERGETICS...According to the HETEROTROPH HYPOTHESIS

If you want to introduce the realm of bioenergetics in a context of their likely evolutionary sequence (simple to complex, and interacting with the environmental changes that are produced), you can present the **Heterotroph Hypothesis**, using the **Narrative** and **Diagram** provided. The diagram is best used on an overhead (or PowerPoint presentation), walking students through the phases from left to right (over time), showing the changes that each phase introduces. If one of your themes is evolution, then this introduction is an excellent way to show how these major components of the chemistry of life could very well have evolved.

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SUMMARY OF BIOENERGETICS PROCESSES
and
THEIR ECOLOGICAL INTERDEPENDENCE

Use the page of diagrams to show the essential features of each process, and to show how they depend upon each other in our ecosystem. This makes an effective review of the bioenergetics unit, and a useful intro to ecology.