



Are you what you eat?

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Making Predictions

Case Study Problem C-643 Intermediary Metabolism

As Rose discovered later, her simplistic understanding of photosynthesis was about half a century out of date. In the 1940's, knowledge of metabolic pathways exploded with the availability of radioisotopes. Carbon dioxide, being one of the simplest compounds of carbon, became one of the first ^{14}C -labeled compounds to be produced and used to study metabolism. [Melvin Calvin](#), a physical organic chemist with broad interests and experience with radioisotopes from the Manhattan Project, exploited CO_2 in a series of elegant experiments to trace the flow of carbon in photosynthesis - work for which he received the [1961 Nobel Prize in Chemistry](#). His major discoveries included the identification the five-carbon molecule that reacts with CO_2 and the reactions dependent on thiamin pyrophosphate that rearrange sugar phosphate skeletons in the conversion of three-carbon intermediates into five-carbon intermediates.

Chlorella pyreniodosa is a unicellular green algae that is easy to grow in aqueous culture. It was used in many early studies on photosynthesis by Calvin. All it needs are light, inorganic ions, and carbon dioxide. The following describes a classic experiment by one of Calvin's associates, James Bassham, on the effects of a transition from light to dark on metabolism by the algae.

After 30 minutes of photosynthesis under steady-state conditions with unlabeled CO_2 , ^{32}P -labeled phosphate was added to an algal culture, and 20 minutes later, $^{14}\text{CO}_2$ was added. Within 10 minutes the Calvin Cycle intermediates had achieved isotopic equilibration but it was not long enough to significantly label the pool of stored carbohydrate (starch). After this equilibration period, the light was abruptly shut off and over the next 15 minutes of darkness, samples of the algal culture were removed at one-minute intervals and killed in ethanol. The lights were turned back on and the sampling continued for another 5 minutes.

The radiolabeled metabolites in the samples taken during the light, dark, and again in the light, cells were extracted and identified by two-dimensional chromatography and the ^{14}C and ^{32}P measured.

1. Draw a time line on the blackboard near your group's table and on it indicate what was done and when it was done in this experiment.
2. Normally only "tracer" amounts of radioactivity are used in these experiments. In other words only a small percentage of the all of the atoms of a given element are actually radioactive. A compound's specific radioactivity is the ratio of radioactive atoms to the total number of atoms in in it. Consider 3-phosphoglycerate, the first metabolite of carbon dioxide fixation produced by the reaction catalyzed by Rubisco. Draw a graph on the blackboard that represents qualitatively how you would predict the specific radioactivity of 3PGA would change during the experiment.



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Interpreting Results

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3. The following figure shows the effects of light and dark on the amounts of ^{14}C and ^{32}P labeling of 3-phosphoglycerate in *Chlorella pyreniodosa*.
- Examine the Figure and, as a group, **make a list** of things you observe in the data - **one clear statement for each observation**.
 - Explain as many of the observations as you can**.
 - Define precisely the aspects of the observations that you cannot explain** as a list of learning issues to guide your preparation for next class.

