

An Investigative Case Study Designed to Promote Critical Thinking Skills

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Notes for Instructor

The end-of-semester project in General Biology at the University of Nevada, Reno, was designed as a coupled-inquiry exercise that promotes higher thinking skills in first- and second-year biology students. Coupled-inquiry is defined by Martin-Hansen (2002) as a two-part process. The first part is a guided inquiry in which the professor puts in place a situation that needs to be investigated. This transitions into an open inquiry in which the students develop their own questions in order to resolve the original problem they were given. The advantage of the coupled-inquiry approach is that it places all students in an equally challenging situation where they must investigate a question that requires them to think critically. Our goal was to develop an independent project that promotes critical thinking and creative thought in our students.

The “Bad, Bad Baby Formula” Case Study

The “Bad, Bad Baby Formula” laboratory is an exercise designed as a case study that sets the stage for students to develop their independent projects. The students are given two different baby formulas, Enfamil with Iron and Carnation Good Start, and they test the protein concentrations of each. The protein concentration given by the manufacturer is known at the start of the experiment and can be found on the formula label. Each team is given a series of baby formula standards that range from 0.2 mg/ml to 1.2 mg/ml in order to generate a protein standard curve. All protein concentrations are generated by conducting a Bio-Rad protein assay (Bio-Rad Laboratories, 2002). The protein concentrations of each baby formula are calculated using the protein standard curve. Students then calculate the percent difference between the actual and observed protein concentrations. Enfamil with Iron usually falls within +/- 15% of the manufacturer’s claim, while the Carnation Good Start falls 50-67% lower than the manufacturers claim. These aberrant results prompt the question, “Why does the protein concentration of Carnation Good Start appear to be ~1/2 the concentration found on the label?”

Preparing Students for the Independent Projects

After initial results and calculations are attained, students are encouraged to derive explanations of their own. The first explanation they commonly propose is that the Nestle' Corporation, which produces Carnation Good Start, has falsified information concerning the amount of protein contained in their product. We then introduce the Infant Formula Act passed by Congress (1980) that requires all baby formula manufacturers to follow strict production guidelines and be closely monitored by the Food and Drug Administration. We then ask the students to look at the labels of the two baby formulas and see if they notice something different about Carnation Good Start. One thing that makes Carnation Good Start unique compared with most baby formulas is the unusual structure of the proteins. The milk proteins in Carnation have been pre-digested into “Comfort Proteins” which are whey proteins that have been broken down into smaller pieces for easy digestion. Carnation digests these whey proteins with small amounts of trypsin. Trypsin is a digestive enzyme found in the small intestines of mammals. Its role is to digest all types of proteins, including whey proteins found in milk products. Students are provided this information and a set of questions to guide them through the critical thinking process (Table 1). Students are expected to formulate a hypothesis and suggest an experiment that could explain why the Bio-Rad assay gave aberrant results in this case.

Table 1. Sample Questions and Statements for Developing a Hypothesis.

<ol style="list-style-type: none"> 1. Have a clear understanding of what a polypeptide chain looks like. For example, can you distinguish between the backbone and the side chains (R-groups)? 2. What are comfort proteins? 3. How does the enzyme trypsin cleave the polypeptide chain? Does it remove a side chain and leave the backbone intact? Have a clear understanding of the mechanism at the molecular level. 4. Have a clear understanding of how the Bio-Rad assay works. 5. Are you able to take all of the information from above and synthesize it into a clear and concise description of what the possible problem may have been when you used the Bio-Rad assay for testing protein concentration in Carnation infant formula?
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In addition, students are directed towards appropriate literature. The first key point that is emphasized is that when scientists “troubleshoot” an experiment, they often do not start with the primary literature. Instead, students are encouraged to explore the rich sources of information that they may never have considered. These include reading textbooks to fill the gaps in their knowledge, calling Bio-Rad or the Nestle’ Corporation to speak with a technical representative, and searching websites. By having this discussion before they begin, we find that students have more time to evaluate their gathered information and don’t spend an inordinate amount of time looking for sources of information.

Part 1 of the Independent Project: The Written Proposal

During the first week of the project, students turn in a rough draft of their hypothesis. While the instructor reads the papers, the students are expected to discuss and share ideas. This provides the students with the opportunity to test their logic and make corrections before having to confront the instructor. After 20 minutes, the instructor re-enters the classroom and is updated by each team. The instructor has each team present its updated ideas and the instructor provides further feedback. The peer-critiquing process commonly leads to teams rethinking or refining their hypothesis, which is an essential part of the critical thinking process.

Based on the first handout and comments from the instructor, students should understand what a basic polypeptide chain looks like and how trypsin cleaves the chain. In addition, students should know what comfort proteins are and how they are made. It also helps for students to know the approximate size of amino acid chains in Carnation Good Start. This information is provided by the representative at the Nestle’ Help Line. An imperative piece of information that students should know are the amino acids that interact with the Coomassie Dye found in the Bio-Rad assay. These two amino acids are lysine and arginine, the same amino acids targeted by the trypsin enzyme. The actual site of dye binding (side chains, backbone, both) is unknown, however, the assay is rarely used to detect small peptide fragments.

After week one, students refine their thoughts and hypotheses and turn in a final draft of their hypotheses with experimental designs included. The majority of students often suggest trying another assay or digesting another baby formula with trypsin to see if similar results occur.

A final note about the first part of this independent project: It is not necessary to dedicate two whole class periods toward discussing the proposal associated with the independent project. The time allotted towards discussion is about one hour per laboratory period. This allows us to conduct other, unrelated laboratory exercises during these two lab classes.

Part 2 of the Independent Project: Conducting Experiments

During weeks 3 and 4, students conduct their proposed experiments. We felt that it was important to monitor teams progress during the actual experiments in order to help students overcome technical difficulties. Even though each team designs its own independent project, to solve the mystery of the Bad, Bad Baby Formula, most teams were using similar laboratory protocols in their projects. Preparation was mandatory and was enforced by requiring teams to prepare flow charts of their experiments. In order to help students overcome minor technical obstacles, we began posting technique tips on the whiteboard and testing students for proper use of a micropipette before beginning their project.

Part 3 of the Independent Project: Scientific Paper and Oral Presentation

On week 5, each team turns in a rough draft of their final paper. During this week, students are encouraged to read over each other's sections and develop an oral presentation from the paper. The final papers are due during week 6 and each team gives a formal presentation on their individual experiments and discussion follows. Use of a coupled format for the independent project helps to stimulate discussion because all teams have been addressing similar questions.

Does anyone know the answer?

After three years of conducting these independent projects, we were able to gather enough information to establish two working hypotheses that could explain the irregular results obtained when the Bio-Rad assay was used to detect protein concentration in Carnation Good Start. The two primary hypotheses are summarized below.

Hypothesis 1:

Trypsin cleaves the polypeptide chain at the carboxyl end of specific amino acids, which includes arginine and lysine. This alters the structure of the polypeptide chain so that amino acids arginine and lysine are now found at the C-terminus of the backbone structure. The partial loss of backbone attachment to these amino acids may prevent the Coomassie Blue dye from binding to these amino acid residues.

Hypothesis 2:

The polypeptides that remain after Carnation Good Start is digested with trypsin may be too small to be detected by the Coomassie Blue dye (Table 2).

Table 2. Average peptide chain length of Carnation Good Start fragments after digestion with trypsin.

Average Chain Length	Percentage Found in Good Start
> 40 amino acids	5%
10-40 amino acids	68%
< 10 amino acids	27%

A team of students digested an untreated baby formula (Enfamil) with chymotrypsin in order to find additional information that could support either hypothesis. Chymotrypsin is a digestive enzyme related to trypsin and is capable of cleaving proteins into small peptide fragments. Unlike trypsin, chymotrypsin cleaves the amino acids tyrosine, tryptophan, phenylalanine, and leucine, and leaves arginine and lysine intact within the peptide backbone. Baby formula digested with chymotrypsin gave aberrant results similar to baby formula that is digested with trypsin. These results suggest that peptide size leads to the anomalous results found in the original Bad, Bad Baby Formula experiment.

Conclusions

The independent project gave students a sense of satisfaction in helping to solve a complex research problem. We feel encouraged by the level of critical thinking skills and overall thought that was put into the project itself. Once students were guided in the right direction, they were able to take the project to new and inventive levels. With a little creativity, the structure of this independent project can be applied to many preexisting laboratory exercises that lend themselves to curious results.

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