## WRITING A LAB REPORT

I find it somewhat humorous that the popular image of scientific process is a lab coat-clad scientist in horn-rimmed glasses shouting "Eureka!" Sometimes it does happen, but usually it is more painful than that. A more truthful image is someone spending months (or years) of reading, reevaluating, and repeating experiments. Even Earnest Rutherford took a good 18 months to interpret the results of the famous gold foil bombardment experiment. Indeed the model of the atom we currently accept took longer than that to develop, and it did not happen from the single experiment in three paragraphs as described in your general chemistry textbook.

The primary means by which scientists develop testable ideas (hypothesis) and their experiments is through reading journal articles. Journal articles are really nothing more than highly developed lab reports, thus your ability to construct an effective lab report is really more about scientific communication rather than simply recording what you have done in lab (the record of lab activity is the lab notebook). It is a formal presentation of your work, and like most formal events, requires you to pay attention to the presentation. Think of it as a "black tie event" where you bring out your best clothes and polish yourself up more than usual.

Lab reports and journal articles follow a fairly set format. One function of this format is to help develop the reader's understanding of the work so they can evaluate the importance and validity of the results. In other words, a journal article or lab report should help the reader understand,

1. The existing body of knowledge (or context) within which the current experiment is being conducted; how the work is significant, why the question being asked is relevant, and the general strategy being used to answer the question.
2. The specific experimental methods and procedures used.
3. The results of those experiments and how they have been interpreted with respect to the existing body of knowledge.

Conveniently, these points of understanding are usually organized into specific sections of a lab report that are described below. Although I go to some trouble to explain the piece of the lab report, I will remind you that reading a lot of journal articles will help you give you practice in seeing how they are constructed. Remember, reading can help you write better.

### *The Introduction*

The first introduction you get to any paper is the title. Titles immediately give you a hint of what is being addressed and are most often are one-line descriptions for the experiment or study. Some simple examples are: Detection of Alcohol Dehydrogenase Activity in Yeast Extracts; Development of a Colorimetric Assay for $NO_3^-$ /$NO^{2-}$; Comparative Study of Booger Mass from North American College Student Populations. Notice that these titles tell you exactly what you can expect to encounter if you read the corresponding paper.

Here is a title and the first few paragraphs from a paper by Y. Matismoto et al. (*Biol. Pharm. Bull.* **27**(3) 422—425 (2004). Read it before I discuss the features of an introduction.

**INHIBITORY EFFECTS OF ESCULETIN ON MELANIN BIOSYNTHESIS**

*Coumarins are widely distributed in plants and are especially abundant in the bark, leaves, and roots of Umbelliferae and Rutaceae plants. So far more than 1300 types of coumarin have been identified as natural or synthesized compounds. Coumarins have recently been reported to have interesting pharmacologic and biochemical properties such as antioxidative,[1,2] antiinflammatory, and antiallergic effects,[3,4] inhibition of platelet aggregation[5] and protein kinase,[6] induction of apoptosis,[7] and antiviral,[8] antidifferentiative,[9] and antimutagenic activity.[10]*

*Melanin pigment is a heteropolymer of indole compounds synthesized within melanocytes in the epidermis. Inhibitory compounds on melanogenesis are useful as*

*skin-whitening agents used in cosmetics and as treatment of hyperpigmentation. Tyrosinase is known to play a critical regulatory role in melanin biosynthesis.[11] Therefore, many tyrosinase inhibitors that suppress melanogenesis in epidermal layers have been actively studied in cosmetics and pharmaceuticals.[12—15] These observations led us to search for naturally occurring tyrosinase inhibitors.*

*Recently, we have isolated esculetin from the seeds of Euphorbia lathyris L. as a mushroom tyrosinase inhibitory compound.[16] To the best of our knowledge, this is the first report of the tyrosinase-inhibitory effects of esculetin. In this study, 18 coumarins and four cinnamic acid derivatives were examined for antityrosinase activity and study of the structure–activity relationship. We evaluated further the inhibitory effect on melanin synthesis in B16 melanoma cells and guinea pig epidermal sheets of esculetin, which showed the strongest inhibitory activity among these compounds.*

This introduction has some good points and bad points. One good point is that it very succinctly summarizes background information and places the reported experiment in context. This paper starts out explaining a little about coumarins—where you can find them and what they do. (It assumes that you know these are small organic molecules.) The second paragraph provides further context regarding melanin formation by the enzyme tyrosinase and how inhibiting tyrosinase appears to be a desirable property in cosmetics. From these two short paragraphs, you, as a reader, have a very good idea of how these experiments are related to other information. This is one of the most important points about a good introduction—it helps you understand why someone went to the trouble of doing the experiment. Will the experiment provide new information? Will it disprove currently accepted models? Will it confirm previously known information or perhaps illustrate that it is also true in a different system? These are the types of questions that are answered in the introduction.

Another good point of this introduction is it explains the general idea or strategy of the work—finding a tyrosinase inhibitory compound called esculetin and comparing it to 22 other ones. Describing an experimental strategy means to briefly outline what types of measurements were performed and how those measurements were taken. Sometimes the introduction for a hypothesis driven experiment (as opposed to a characterization experiment like the one in the example) will also explain what and why certain results were expected.

An introduction should provide the reader with,

1. background information explaining why the experiment was done, and
2. a summary of the experimental system or techniques used in the work.

The bad point about this introduction is the authors don't seem to point out that esculetin is a coumarin and that this class of chemicals are tyrosinase inhibitors. But by the end, you can kind of guess that this is the case.

You should also note that there are 16 cited references in these three paragraphs. As in most published articles, the bulk of the introduction is background information coming from outside resources or previous work. While it is true that you are usually undertaking a lab because you don't want to fail the class, I expect you to imagine beyond the immediacy of a lab assignment and think about why this lab could be interesting. Someone once told me that anything can be interesting, you only have to decide that it is. Here is your opportunity to discover that what you are doing could be important or is at least relevant to someone else. Take a few minutes and look up something about the lab or experimental system you are using. You might find that things are more interesting than they appear.

***The Material and Methods Section***

Here is an excerpt of the materials and methods from the same paper.

***Assay of Tyrosinase-Inhibitory Activity*** *The assay was performed as previously described.[16] One milliliter of a 1.5mM L-3,4-dihydroxyphenylalanine (DOPA) solution, 0.1 ml of dimethyl sulfoxide (DMSO) with or without sample, and 1.8 ml of 1/15 M*

*phosphoric acid buffer solution (pH 6.8) were mixed. The mixtures were preincubated at 25 °C for 10 min. Then, 0.1 ml of the aqueous solution of mushroom tyrosinase (1000 U/ml, Sigma Chemical Co., St. Louis, MO, U.S.A.) was added, and the reaction was monitored at 475 nm. A control reaction was conducted with DMSO. The percentage of inhibition of tyrosinase activity was calculated as follows: inhibition (%)=(A-B)/A x100, where A represents the difference in the absorbance of the control sample between the incubation time of 0.5 and 1.0 min, and B represents the difference in the absorbance of the test sample between the incubation time of 0.5 and 1.0 min. The results were the mean of the three concurrent readings.*

***Inhibitory Effect on Melanogenesis Using Cultured B16 Melanoma Cells*** *B16 murine melanoma cells were cultured in Dulbecco's MEM medium supplemented with 5% heat-inactivated (56 °C, 30 min) fetal bovine serum at 37 °C in a humidified atmosphere containing 5% $CO_2$. Ten microliters of DMSO with or without esculetin was added to the culture medium (10 ml) 24 h after cell seeding (2.0 x $10^4$ cells/90-mm dish). After 6 d of culture, the cells were harvested and the cell counts were determined. The degree of whitening of pelleted cells was observed with the naked eye. Melanin content was measured using a modified version of the methods of Oikawa and Nakayasu[17] and Hosoi et al.[18] Approximately 5x$10^6$ cells were pelleted by centrifugation at 1500x**g** for 5 min and then washed twice with phosphate- buffered saline. After further centrifugation, the supernatant was removed by carefully decanting and the precipitated cells were solubilized with 1 ml of 1 M NaOH at 80 °C for 30 min in a capped test tube. The absorbance was measured at 400 nm, and the melanin content per cell was calculated. Data are expressed as a percentage of control, and are mean values±S.D. Student's t-test was used for the statistical analysis of the data.*

Materials and methods sections are written in a style that is very economical and terse. The greatest challenge is to decide what information should or should not be included. The general rule of thumb for this decision is to include enough detail so someone of your ability could repeat exactly what you did. This requires that you recognize what is general knowledge and what is specific knowledge. In the above example, the phosphate buffer used in the assay would be general knowledge. They don't describe how they made it, only its concentration and pH ($^1/_{15}$ M is rather odd). The incubation temperature and time are specific knowledge and are defined. There is also no mention of any the results or why they did the experiment, just a matter-of-fact statement of how they performed the procedure using the terms that other scientists will be familiar with.

You will notice that this example does not contain personal references (i.e. no pronouns like I, me, we, etc.). The experiments were obviously performed by a person, yet there is never any mention of "I did this, then we did that." Furthermore, everything is in past tense, because the procedures have already been performed. We find this to be the case when referring to previously established results (i.e. citing someone else's work) in the introduction.

There are some general characteristic that are worth mentioning about the style of writing for the materials and methods section.

1. Write the information as a narrative, not as steps in a recipe. The important thing is that you avoid statements like *"0, 50, 100, 150, 200 ul of 50 mM urea stock was placed in tubes and 800, 850, 900, 950, and 1000 ul of buffer was added."* Everyday procedures like measuring or diluting or pouring are simply understood to have been used and you can rely on your reader having some common sense (usually).

2. The narrative is not presented chronologically, it is simply broken into sections that refer to either a specific experimental procedure, or an experiment performed with a set of samples. Don't fall into the trap of "First I grew the cultures. Then I purified the plasmid…"

3. The procedures contain lots of specific details and not general guidelines. Concentrations, times, volumes, and temperatures are defined explicitly. Don't say things like "Solutions were incubated in a hot water bath". "Solutions were incubated at 60 $^{o}$C for 5 min." is much more informative.

4. It is perfectly appropriate to reference a commonly used procedure and to simply provide ranges of concentrations used in the procedure. For example, "Ammonia concentrations were determined using the urease-Berthelot assay (Fawcett and Scott, 1960), and compared against a standard ammonia curve ranging from 0-125mM ammonia ($R^2$ = 0.998)". In this case, you are simply saying that you used a published procedure for a given but giving specific range over which the assay worked.

I know this is a lot to remember, but the materials and methods section is usually what causes students the most problems. I will point out that the more you read published papers, the easier it becomes to figure out how to organize your own writing. I have included some actual student examples borrowed from an OSU website to give you practice in distinguishing strong materials and methods statements from weak statements.

*Weak* Distillation fractions three and four were combined in a 100ml round-bottom flask. To this flask was added 1.966g (0.0114 mol) of benzoic acid. The flask was then connected to a long column, distilling head, and condenser. Glass-wool and foil was again wrapped around the column and distilling head.

*Strong* **In a 100 mL round bottom flask equipped with a water jacketed condenser and wrapped column and head, 1.966 g (0.0114 mol) of benzoic acid were added to the combined third and fourth fractions.**

*Weak* Next, the copper solution was prepared by weighing out 0.1821 g of copper nitrate and dissolving it in 10mL of tap water.

*Strong* **Then, 0.1821 g of copper nitrate was dissolved in 10mL of tap water**.

*Weak* The contents of the flask were poured into a separatory funnel, and mixed with increasing vigor. The evolving gas was vented periodically. The product was allowed to separate into two distinct layers. The bottom, aqueous, layer was bright yellow. The top, ether, layer was reddish-brown and transparent. Each layer was decanted into separate containers.

*Strong* **The product was transferred to a 1 L separatory funnel and upon mixing separated into a bright yellow solution (bottom layer) and a clear ethereal reddish-brown layer with the evolution of carbon dioxide.**

***The Results and Discussion Section***

Again, here are a few paragraphs from the Results and Discussion section of our example paper. Pay attention to the types of information and statements that are made.

> *Coumarins have many interesting pharmacologic and biochemical properties. However, there are few reports on the inhibitory effect of coumarins against melanogenesis and there has been no systematic research. First, to investigate the structure–activity relationship of coumarins, the $IC_{50}$ values on mushroom tyrosinase of 18 coumarins and four cinnamic acid derivatives were measured. Table 1 summarizes the $IC_{50}$ values of test compounds used in this study. Among these compounds,*

*esculetin, which has hydroxyl groups at the C6 and C7 positions of the coumarin ring, exhibited the strongest inhibitory activity ($IC_{50}$=0.043mM), followed by umbelliferone ($IC_{50}$=0.42 mM), which has only one hydroxy group at C7. Daphnetin, scopoletin, and 6,7-dihydroxy-4-methylcoumarin showed a weak inhibitory effect and the other compounds were ineffective.*

*We performed further studies to estimate the in vitro inhibitory effects of esculetin on melanogenesis. The inhibitory effects of esculetin on melanogenesis in cultured B16 melanoma cells are shown in Figs. 1 and 2. The growth rate of B16 melanoma cells was not significantly altered after 6 d of incubation in the 1.25 to 5.0 μM concentration range of esculetin, indicating that the esculetin-induced inhibitory effect on melanogenesis in B16 melanoma cells occurred without affecting cell proliferation. After 6 d of incubation with esculetin 5.0 μM, the melanin content decreased to 65.5% compared with that in control cells (=100%), while esculetin 1.25 and 2.5 μM did not affect melanin production (Fig. 1). The results are consistent with the visible appearance, as shown in Fig. 2. At the cellular level, we were unable to assess the inhibitory effect on melanogenesis at the more effective concentration because esculetin exhibited strong cytotoxicity. Therefore, we examined inhibitory effects of esculetin on melanogenesis using split-epidermal sheets from black guinea pigs…*

The first major point to notice is that in the first paragraph they highlight important results, they don't explain the details of everything. Nevertheless, all of the data is presented in a table (not shown here), but they use the narrative of the paper to emphasize the compounds that were the most inhibitory as well as those that were the least inhibitory. Furthermore, they explain why they measured the $IC_{50}$ values (to "investigate the structure-activity relationships of the coumarins"). Thus this first paragraph summarizes the rationale and the results that came from one experiment.

The second paragraph is a bit different in its content. An early statement about the fact that esculetin did not affect growth rates is important because it tells you that reduction of melanin synthesis was caused by inhibition of the process, not from some other effect associated by killing the cells. In addition, they outline the fact that the only noticeable effect of esculetin was at the highest concentration used. They then note a limitation of this study due to cytotoxicity and go on to explain an alternative experiment to address this limitation. This is a good illustration of what it means to interpret experimental results and to explain the rationale behind the experiments.

Generally we include all of the ***meaningful*** data coming from your experiments within a Results and Discussion section. "Meaningful" not only means data supporting your argument (i.e. different from the control), but also data that disproves your argument (i.e. *not* different from the control). "Meaningful" also alludes to the fact that the data were the result of real experimental differences, not because you goofed something up. Perhaps a useful, but crude, test of whether something should be included is to use the two rules below.

- ***The "Aha!" Rule***. If you observe a result or gain some understanding that stands out and you say to yourself, "Aha! Now I get it!", that information should be included. (Include things that show you to be clever and observant.)
- ***The "Awe, Shit!" Rule***. If you totally misunderstood a direction, or measured something incorrectly, or discovered a mistake in your calculations and say "Awe, shit!", then you can probably omit it from the report. (You generally don't want to reinforce the view that you are a total bonehead.)

Remember that the primary function of a lab report is communication, which means you are transferring your knowledge and insight about the experiment to someone else so they can critically evaluate whether or not they agree with you.

***The Conclusion***

There isn't always a separate Conclusion section in a lab report; sometimes there is simply a concluding paragraph. You can decide what method suits your style, but as with any written piece, you need to have some sense of closure signaling the reader that you have make your case. Often there is some indication of further work to be undertaken or other areas to apply the new information to. In our example paper, the authors did not have a separate conclusion but just included this closing paragraph in the Results and Discussion. Notice that they define the extent to which their work can be interpreted and include some references to other information that helps to support their general conclusions.

> *… Although the exact antityrosinase mechanism of esculetin could not be elucidated, we have reported that esculetin exhibited competitive inhibition of DOPA oxidation by tyrosinase.[16] In addition, antioxidants such as α-tocopherol and ascorbic acid derivatives are generally used as active agents to inhibit melanogenesis in epidermal melanocytes.[14,21] Therefore, it is considered that the antioxidative activity of esculetin[1,2] can also inhibit oxidative polymerization of various intermediate products derived from DOPA during melanin formation in melanocytes. However, further detailed studies are necessary to understand the inhibitory mechanism of esculetin.*

The sense I get from this last sentence is that the results of this study should be viewed as a first step to understanding exactly how esculetin inhibits melanin formation. I don't think there is much question that it does, but the authors are careful to point out that esculetin "*can also inhibit oxidative polymerization of various intermediate products derived from DOPA during melanin formation in melanocytes"*. This means that they are assuming esculetin acts the way it does based on logical deduction from several pieces of evidence including the experimental results reported in their paper. Nevertheless, they simply do not have specific experimental evidence that esculetin directly interferes with melanin formation…yet.