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"It was a cold and rainy night": Set the Scene with a Good Introduction

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In theatrical productions, there is a process called *setting the scene*, which is the act of describing a situation so that the audience understands what is happening. Setting the scene lays the groundwork for what to expect during the remaining acts in the production. Similarly, a well-written introduction in a scientific paper sets the scene for the reader. It starts by telling the reader what is happening or has happened (the context), and ends by giving the reader a glimpse of what follows in the remainder of the article (the plot).

Introductions seemingly should be easy to write, since they do not require details about methods and results or a discussion of the results. Besides, the introduction is usually found right after the abstract, where you already summarized the content for the reader. In actuality, however, writing a good introduction requires considerable time and thought. Here I provide information about the structure of a good introduction and how to avoid common problems that editors see with submitted manuscripts.

The Conical Introduction

Introductions have shapes. Some individuals see them as funnels, others as cones or inverted pyramids. Whatever image you choose should go from large to small, broad to narrow. This is how the information in the introduction should flow as well (Fig. 1). Begin by providing the reader with background information on the topic of the paper. Describe what is known about a disease, technique, or compound and why it is an important topic. Do not be concerned if this takes several sentences. If there is a certain amount of background information that you believe the reader must have to follow the remainder of the article, include it. But make sure that the background information directly relates to your specific study. For example, if you are reporting on a new marker for pancreatic cancer, do not devote unnecessary text to the epidemiology, therapy, life expectancy, medical costs, etc., of cancer in general. Get to the known information about pancreatic cancer as soon as possible.

Having presented relevant background information, the next step is to narrow the introduction and focus the reader's attention on the importance of continued research on particular aspects. Tell the reader about needed but unknown information, an unsolved problem, a knowledge gap, or limitations of prior studies. There may be a lack of a good analytical technique or the availability of a new animal model. Perhaps no one recognized the problem before now or tied the literature together to identify a possible solution. The important goal here is to demonstrate to the reader that there are important missing pieces of the puzzle that need to be filled in. Using the analogy of a theatrical production, you should set the scene by putting the necessary background information into the proper context.

Now the introduction can be narrowed again by focusing on the goal of your study (the plot). From this point on, the text must provide a clear rationale for why you initiated the study. The reasons for doing research are limited. You test a hypothesis, answer a question, solve a problem, or fulfill a purpose. The text should include something like the following:

- *We hypothesized that . . .*
- *We tested the hypothesis that . . .*
- *We asked whether . . .*
- *To answer this question, . . .*
- *This prompted us to investigate whether . . .*
- *To resolve this apparent difference . . .*
- *We solved this problem by . . .*
- *The purpose of our study was . . .*

Importantly, this type of presentation tells the reader to expect a clear answer by the end of the article regarding the study goals or hypothesis—i.e., true/false, yes/no, works/doesn't work.

Optionally, some writers choose to add a short concluding sentence or two telling the reader something about the approach taken, the plan of attack, or the proposed solution in the paper and its importance. If included, however, my recommendation is that you not provide method details, results, or conclusions. The reader should already have had a brief exposure to these items through the abstract.

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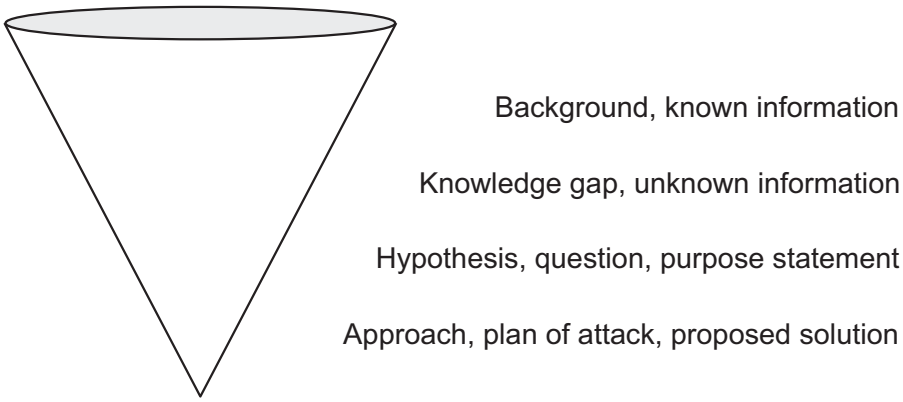

Fig. 1. The introduction: a cone or funnel.

Example 1 provides the introduction for a hypothetical study of a biomarker in vascular inflammation. Compare the format of this introduction to the cone concept in Fig. 1. The first sentence (top of the cone) tells the reader that the study relates to the broader topic of cardiovascular disease, which is an important health problem. The next sentence narrows the topic to chronic inflammation, which is linked to cardiovascular disease, followed by a sentence that focuses the topic further to β-selectin, a marker of inflammation that is increased in the serum of patients with peripheral vascular disease. Besides referenced association studies,

Example 1

Cardiovascular disease is a public health problem worldwide. Chronic inflammation has been linked to cardiovascular disease and sudden cardiac death *(1–3)*. Recent studies have demonstrated that a strong association exists between β-selectin, a recognized systemic marker of inflammation, and cardiovascular disease *(4–6)*, and that patients with peripheral vascular disease have increased serum concentrations of β-selectin that correlate with the degree of functional impairment *(7,8)*. Additionally, prospective studies have presented data regarding the prognostic value of β-selectin in predicting the severity of underlying cardiovascular disease and risk of mortality. The Vascular Inflammation Prediction (VIP) Study found a positive correlation between concentrations of β-selectin and the risk of developing cardiovascular disease *(9)*. The Canadian All-Cause Mortality Study revealed that individuals with serum β-selectin concentrations >90 μg/L are 4.5 times as likely to die within 5 years as those with concentrations ≤90 μg/L *(10)*.

Whereas these association and prospective studies indicate that β-selectin is a predictor of cardiovascular disease and risk of mortality, they provide little information about the underlying pathophysiology of vascular inflammation and the contributory role, if any, of β-selectin.

We therefore investigated in an animal model whether β-selectin is a cause or just a marker of vascular inflammation associated with cardiovascular disease.

Using a herpes simplex virus type 2 infection protocol to stimulate continuous β-selectin production in mice, we investigated the effects of β-selectin production on the development of atherosclerotic lesions, life span, and potential mechanisms of β-selectin–induced inflammation.

Example 2[1]

Because of the speed and selectivity that it affords compared to other techniques, liquid chromatography (LC) coupled with electrospray ionization–tandem mass spectrometry (ESI-MS/MS) is being increasingly used in clinical laboratories to quantify steroids *(1)*, therapeutic drugs *(2–4)*, vitamins *(5)*, biogenic amines *(6)*, and metabolic intermediates *(7–9)*. One use of LC-ESI-MS/MS in our laboratory is for quantifying immunosuppressants in whole blood and serum. We use methanol for sample preparation and chromatography because it is readily available and less costly than acetonitrile. Methanol is a mobile-phase component in multiple published methods for immunosuppressants *(2, 10–14)*.

When quantifying immunosuppressants by this approach, we encountered the problem of a slow loss of 32-desmethoxyrapamycin, the internal standard for sirolimus, if the methanolic working solution was stored at ambient temperature. We presumed that this loss resulted from degradation of 32-desmethoxyrapamycin in the methanol being used, an effect similar to that reported for the internal standard ascomycin in some brands or grades of acetonitrile *(15)*. In the course of investigating whether alternate commercial sources and grades of methanol would correct the loss of 32-desmethoxyrapamycin, and also be suitable for use in the mobile phase, we noted large differences in the ionization of not just 32-desmethoxyrapamycin, but also other immunosuppressants and their internal standards when different sources and grades of methanol were evaluated.

Coeluting components originating from biological matrices have previously been shown to negatively affect (ion suppression) or positively affect (ion enhancement) the analyte signal in ESI-MS analyses. This report describes the phenomenon on ionization changes related to the organic solvent used in the LC-ESI-MS/MS analysis.

[1]Modified from Clin Chem 2007;53:1827–34.

the introduction next emphasizes that 2 major prospective studies have found a positive correlation between β-selectin and actual cardiovascular risk. The first paragraph has provided background and known information from citable work, all the way demonstrating to the reader the importance of β-selectin as a subject of research. The second paragraph (narrower section of the cone) presents the unknown information (knowledge gap) that previous work has failed to address. Even without the question being explicitly stated, the reader can begin to deduce what the study question will be. The third paragraph (even closer to the tip of the cone) narrows the focus to the question itself and the purpose of the study. Is β-selectin a contributing factor or just a marker of cardiovascular disease? At the minimum, there will be a yes/no answer. The last paragraph of the introduction gives the reader some clue as to how the study was performed, i.e., using a viral infection model in mice. No method details are provided, no results provided, no conclusions stated. Overall, this introduction follows the model in Fig. 1.

Transition Phrases

In the introduction, the story becomes clearer if transition phrases are used. Transition phrases allow the author to emphasize important points, and also help the reader differentiate the known, the unknown, the question, and the experimental approach. I previously listed some examples of ways to lead into the question or hypothesis. Examples of transition phrases that can be used to highlight the known, or link the known to the unknown, are shown below:

- *These prior studies show that . . .*
- *Supporting the theory that . . .*
- *These studies are important because . . .*
- *Interestingly, . . .*
- *More importantly, . . .*
- *Using this information, . . .*
- *Yet, . . .*
- *Unlike . . .*
- *Whereas it has been shown that . . .*
- *On the other hand, . . .*
- *It is unclear . . .*
- *The question remains, however, . . .*
- *Although prior studies demonstrated . . .*

Different Study Types, Same Model

Many published articles describe a new method or a secondary finding that sheds new light on a topic.

These types of studies were not initiated to directly answer a question or test a hypothesis, yet they had a purpose that should be described in the introduction. Regardless of the type of study, the same process of honing down to the problem to be addressed, as illustrated in Fig. 1, can be followed when writing the introduction. Example 2 and the learning exercise at the end of the article illustrate ways that this can be done. Example 2 is a modified introduction from an article describing the finding that, in addition to biological matrices, solvents can impact the performance of mass spectrometric assays. The introduction starts with the broad topic of electrospray ionization mass spectrometry, describing what advantages it has and how clinical laboratories are successfully using this technique, subsequently narrowing the subject to a specific assay that served as the origin for the study. This is the known information. The second paragraph, while not directly describing a knowledge gap or problems with previous studies, brings a previously unknown problem to the attention of the reader. It also indirectly introduces the question: Does the quality of solvents have any significant impact on ionization efficiency in electrospray mass spectrometry? The last 2 sentences (paragraph 3) close the introduction by stating the purpose of the paper and what new information is going to be provided. This introduction, although different in style, narrows from known information to a previously unknown problem to the specific purpose of the paper.

Length, Detail, and Overlap

Introductions tend to be too long rather than too short. A long introduction reminds me of the courtroom scene in a television show, where an attorney keeps feeding statements to a witness until the frustrated judge asks, "Counselor, is there a question in there somewhere?" Similarly, in sifting through what was intended to be an impressive overview of the topic and the issues, the reader may fail to appreciate the question when it finally arrives.

There are several ways to avoid giving too much information. One is to characterize the audience of the selected journal. Ask yourself, "If I were the reader, how much information would I really need to understand the study question and why it matters?" Another way to avoid excessive length is to go back in time only as far as needed to bring the reader up to speed. Unless being cited as influential work in the field, is mention of older work or an older reference necessary? A third way is to set a target word limit before linking the known information to the unknown information, and then similarly the unknown information to the study question. The fourth way is to consider whether some of the information or associated references might fit better into the discussion section, where you are interpreting your results and their relevance.

The last option above calls attention to a couple of problems that editors encounter in submitted papers: (*a*) unnecessary overlap of the introduction and discussion sections and (*b*) inconsistencies between these 2 sections. A few brief sentences at the beginning of the discussion help reorient the reader to the purpose of your study and your findings, but you should try to keep background or reference material in one section or the other, not both. Repetition between the sections not only wastes words, but also can create the impression that you had little to discuss in the paper and thus reused background information to fill space. As you try to interpret your study results and put them in context with other studies, you may find that some background or reference material fits better in the discussion than in the introduction. This gives you the opportunity to link specific results or points of discussion to others' work, citing their work where it makes most sense.

Consistency with Other Sections

Although you want to minimize repetition, it is important that the text be consistent among all of the sections of the paper. Background information, knowledge gaps, purpose statements, and proposed solutions in the abstract should be consistent with the introduction. The methods used must reflect any mentioned in the introduction. The results must relate to the study question, hypothesis, or problem first presented in the introduction. The discussion, or summary if separately written, must answer the question posed in the introduction. Sometimes reviewers and editors request changes to the text, restatement of the question or problem, reinterpretation of results, or modification of the conclusions. Thus, it is a good idea to take a fresh look at the introduction after the final draft is written or after any revision to be certain that it is still accurate and consistent with the rest of the article.

Learning Exercise

Below I have provided 10 sentences that together make up an introduction for a paper describing a new method. Using the concept for writing an introduction shown in Fig. 1, rearrange the sentences to create an introduction. Compare your final product with the one provided in the box after the list of selected additional reading materials.

- Iohexol is not bound to serum proteins and is filtered through the glomerulus, with no identifiable reabsorption or tubular secretion, making it an ideal marker for estimating GFR.

- Ultraperformance liquid chromatography (UPLC), a recently introduced modification of LC, allows rapid chromatography owing to faster gradient curves, as well as the potential to use smaller particles and higher flow rates.
- Protocols have been developed that involve a single intravenous injection of iohexol followed by timed blood collections.
- Iohexol is an iodinated contrast dye that has been shown to be useful in clearance studies for the determination of GFR.
- We have combined these 2 techniques to develop a UPLC-MS/MS assay for iohexol in human serum that uses a simple sample preparation, a structural analog internal standard with the same retention time, and a ballistic gradient for rapid chromatographic analysis.
- Both of these techniques require lengthy run times to separate iohexol from endogenous interfering compounds and the internal standards.
- In the subset of patients with suspected renal insufficiency for whom it is important to have an accurate assessment of glomerular filtration rate (GFR), clearance measurements provide the best information.
- No urine collection or quantification in urine is necessary, an advantage over iothalamate, the other agent used for GFR studies.
- By comparison, the high selectivity of tandem mass spectrometry (MS/MS) as a detector generally allows simpler specimen cleanup and shorter chromatographic times compared with UV detection.
- The majority of published methods for quantifying iohexol have used capillary electrophoresis or gradient elution liquid chromatography (LC) coupled with ultraviolet (UV) detection.

Final Thoughts

When you introduce an important speaker, you want to give a "proper introduction." This usually entails telling the audience about the speaker's background, area of research, and the topic to be presented. If you go on and on about the speaker's background and accomplishments, or spend too much time talking about how you came to know this individual, or forget to reinforce the topic of the lecture, by the time the speaker says a word the audience may have trouble recalling why they were there in the first place. We have all suffered through such introductions. Your own work must be important to you; otherwise you would not want others to read it. So give it a proper introduction as well, using the tips and ideas that have been presented.

Suggested Additional Reading

Friedman GD. Please read the following paper and write this way! Am J Epidemiol 2005;161:405.

Katz MJ. From research to manuscript. New York: Springer, 2009.

Zeiger M. Essentials of writing biomedical research papers. New York: McGraw Hill, 2000.

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Answer to Learning Exercise[1]

In the subset of patients with suspected renal insufficiency for whom it is important to have an accurate assessment of glomerular filtration rate (GFR), clearance measurements provide the best information. Iohexol is an iodinated contrast dye that has been shown to be useful in clearance studies for the determination of GFR *(1)*. Iohexol is not bound to serum proteins and is filtered through the glomerulus, with no identifiable reabsorption or tubular secretion, making it an ideal marker for estimating GFR. Protocols have been developed that involve a single intravenous injection of iohexol followed by timed blood collections *(2–4)*. No urine collection or quantification in urine is necessary, an advantage over iothalamate, the other agent used for GFR studies.

The majority of published methods for quantifying iohexol have used capillary electrophoresis or gradient elution liquid chromatography (LC) coupled with ultraviolet (UV) detection *(5–9)*. Both of these techniques require lengthy run times to separate iohexol from endogenous interfering compounds and the internal standards. By comparison, the high selectivity of tandem mass spectrometry (MS/MS) as a detector generally allows simpler specimen cleanup and shorter chromatographic times compared with UV detection. Ultraperformance liquid chromatography (UPLC), a recently introduced modification of LC, allows rapid chromatography owing to faster gradient curves, as well as the potential to use smaller particles and higher flow rates. We have combined these 2 techniques to develop a UPLC-MS/MS assay for iohexol in human serum that uses a simple sample preparation, a structural analog internal standard with the same retention time, and a ballistic gradient for rapid chromatographic analysis.

Comment: Following the cone shape model, this introduction narrows from known information to a problem to a solution to the problem. The first paragraph provides a general overview of iohexol and why it has advantages in GFR evaluations. The second paragraph narrows the focus to published methods for quantifying serum iohexol and their drawbacks. Although unknown information or an unsolved problem is not directly stated, the need for an improved assay is implied. The introduction closes with a proposed solution to the problem. Is there a need to propose a hypothesis or ask the question of whether mass spectrometry could be used to quantify iohexol? One could do so, but the answer must be yes, otherwise there would be no need to report the new assay.

[1] Modified from Clin Chem 2009;55:1196–202.