# Annotated Writing Examples

Please use these examples along with the writing text and the guidelines posted on the course website as you prepare portions of your papers. Please keep all materials related to your writing assignments in a notebook, including returned papers and instructor's comments. Go through the following steps for each new assignment.

- look over the relevant examples and write down general conclusions
- compare your notes with general guidelines (on line, writing text)
- look at the specific recommendations for the subject of the paper
- start preparing your own piece

For example, your first assignment will be to complete a materials and methods section. Based upon the introductions to the published articles what would be an appropriate style? How do authors typically use verb tense? What kinds of information do they present? Good scientists are not necessarily good writers. What mistakes did the author(s) make, if any?

Read the recommendations for the first assignment and write it up. When the comments come back compare them with the notes you took and apply your experience to the next assignment.

**Caveats**

The features of effective writing are universal. However, specific rules for technical writing not only vary among different journals and different disciplines, but they also change over time. Journals set requirements for form and style in order to maintain consistency. The examples used here are of the more common styles currently in use in biological journals. There are exceptions to the "rules." For example, the journal Science combines sections so that there are just three parts, namely abstract, body of the paper, and references.

You are not expected to use formal citations for your introductory level papers, nor does your writing have to be as unreadable as some of the sample passages will probably seem to you. The authors wrote for an informed readership, namely people who work in their field and are well aware of technical terms and abbreviations, and the significance of the research. In general, truly effective writing is clear and understandable to a broad readership.

Portions of the examples have been deleted for the sake of brevity. Deleted passages are marked with three periods (...).

## EXAMPLES FROM MATERIALS AND METHODS SECTIONS

### *Item #1* Magnet-induced disorientation in hatchling loggerhead sea turtles

William P. Irwin and Kenneth J. Lohmann, *J Exp Biol 206: 497-501, 2003*

**Animals**

Hatchling loggerhead sea turtles, *Caretta caretta L.,* were obtained from nests at a beach hatchery in Hillsboro, FL, USA. Nests at this hatchery had been relocated from the Fort Lauderdale and Pompano Beach areas within 24 h after being deposited ...

**Experimental arena**
Experiments were conducted in a black, plastic, circular pool 1.7 m in diameter and 0.5 m high. The pool was filled with water to a depth of 0.3 m and was enclosed by a removable lightproof cover (Fig. 1). Observers dark-adapted for 30 min or longer were unable to perceive any light through the cover ...

**Procedure**
For each trial, a hatchling was placed in a Lycra harness that encircled the carapace but did not inhibit swimming movements (Salmon and Wyneken, 1987). Turtles were assigned to one of two groups. One set of turtles (N=13) had a 24 g, 14 mm SpinBar® magnet attached by Velcro to the harness on the dorsal side of the turtle approximately 1 cm posterior to the nucal scute ... continuously monitored the heading of the turtle throughout each trial.

All trials were conducted between 20:00 h and 01:00 h, the period when most loggerhead hatchlings emerge from their nests ...

**Data analysis and statistics**
The data-acquisition computer calculated the mean heading for each turtle based on all data collected during the final 60 min of the trial (i.e. the period beginning 3 min after the light was turned off). The orientation of each group of turtles was analyzed using a Rayleigh test and the distributions of the two groups were compared using Watson's U2 test (Batschelet, 1981).

---

The purpose of a methods section is to succinctly document the methodology so as to make it reproducible.

Note the organization of this example, which separates materials and methods by category rather than going through one experiment at a time sequentially. In fact, methodology and experimental design are not the same thing. A methods section does not describe the objective of an experiment, why specific steps were carried out, or the way in which a hypothesis was tested. It is strictly for reporting methods that were employed in the laboratory or in the field.

It appears at first that irrelevant information was included, that is, does it really matter that the pool was black in color? Actually in this case it did matter. Exposure of the turtles to light was to be very carefully controlled, and the black color prevented reflections that would compromise the experiments. On the other hand, it would have been silly if the authors had stated that the turtles were collected using red plastic buckets, or that they used a particular computer program in order to conduct the analysis.

## *Item #2* Characterization of the signal that directs Bcl-xL, but not Bcl-2, to the mitochondrial outer membrane

Thomas Kaufmann, Sarah Schlipf, Javier Sanz, Karin Neubert, Reuven Stein and Christoph Borner, *J Cell Biol 160: 53-64, 2003*

**cDNAs and site-directed mutagenesis**
The cDNAs for human and mouse Bcl-2, human Bax, FLAG-tagged human Bcl-2, and Bcl-xS and FLAG-tagged Bcl-xS devoid of the last 21 amino acids (FLAG–Bcl-xSTMB) were generated and subcloned into the pcDNA3 or pcDNAampI vectors (Invitrogen) as previously described (Borner et al., 1994; Conus et al., 2000a; Lindenboim et al., 2000). The cDNA for human Bcl-xL was obtained from G. Nunez (University of Michigan, Ann Arbor, MI) (in pcDNA3). pEF-Bcl-xL–FLAG-puro was a gift from D. Huang ...

**Protein expression and subcellular fractionation**
Human embryonic kidney cells (HEK293) were grown on 100 mm until 80% confluent and then transfected with 10 $\mu$g of plasmid DNA using 25 $\mu$l of Superfect (QIAGEN) as described by the manufacturer. After 3–6 h, the Superfect–DNA complexes were removed and the cells were cultured for another 42 h. ...

**Submitochondrial fractionation**
Fractionation of mitochondria isolated from rat liver or HEK293 cells into inner membrane, outer membrane, and matrix components was performed exactly as previously described (Hoppel et al., 1998). ...

**Sodium carbonate extractions**
Crude mitochondria or microsomes (as prepared above) were resuspended in 0.1 M sodium carbonate (pH 12) and incubated for 20 min on ice. After centrifugation, the supernatant (containing the alkali-extractable proteins) was titrated to neutral pH with HCl. The pellet (containing the alkali-resistant fraction) was washed three times ...

**SDS-PAGE and Western blotting**
30 $\mu$g of protein from subcellular fractions and sodium carbonate extractions were run on 15% SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane (BDH), and immunodetected by anti-h/m/rBcl-x (S-18, 1:2,000; Santa Cruz Biotechnology, Inc.), anti–hBcl-2 (clone 124, 1:1,000; DakoCytomation) ... CONTINUED

---

The authors saved a lot of space by providing a reference for the submitochondrial fractionation procedure instead of reporting it in full. You can save space in your own papers by describing methods by name and citing a source. Your materials and methods for the protein lab can be made very succinct that way.

Again, methods are organized by type rather than sequentially. The former style is much more efficient.

The authors found it convenient to describe sources of materials within the description of methods rather than listing them separately, which is often the more effective way to do it.

Specialized chemicals and specialized instruments and sources for them are identified when using a specific brand, lot, supplier, etc. is critical to the success of the project. Note that the authors did not describe such common lab items as pipettors, glassware, centrifuge tubes, vials, microscope slides, etc. nor did they describe such instrumentation as centrifuges, spectrophotometers, etc. We don't describe such items when their use is implied by the methodology that is described. For example, to incubate crude mitochondria on ice the authors obviously needed a bucket of ice.

Note how the composition of MSH buffer was described. This is standard notation for describing solutions.

## *Item #3* Localization of pendrin in mouse kidney

Susan M. Wall, Kathryn A. Hassell, Ines E. Royaux, Eric D. Green, Judy Y. Chang, Gregory L. Shipley, and Jill W. Verlander, *Am J Physiol Renal Physiol 284: F229-F241, 2003*

*Animals*. Nonalbino Swiss mice weighing 20-30 g were studied (Harlan, Ardmore, TX). Mice consumed a balanced rodent diet (Zeigler Brothers, Gardners, PA) and tap water. Mice were anesthetized with 100% O2 at 1 l/min with 4% isofluorane before death.

*Dissection of tubules*. Mice were injected with 1.5 mg furosemide ip 30 min before death. The kidney was perfused initially with 10 ml of ice-cold dissection solution and then with 20 ml of the same solution containing 1 mg/ml collagenase B (0.2 U/mg; Roche, Indianapolis, IN) and 1 mg/ml BSA (Sigma). The dissection solution contained (in mM) 144 NaCl, 5 KCl, 1 Na2HPO4,

1.2 MgSO4, 2 CaCl2, 5.5 glucose, and 10 HEPES, pH 7.4. The kidneys were removed, and a coronal section was made that contained the entire corticopapillary axis ...

*Preparation of total RNA from kidney slices*. After death, the left kidneys were excised from the mice and coronal slices were made. Each slice was cut into three regions: cortex, outer medulla, and inner medulla. Each piece was snap frozen in liquid nitrogen and then weighed. Isolation of total RNA was performed by using an RNeasy minikit (Qiagen). The kidney tissue was placed in RLT buffer (20 ml buffer/g kidney tissue) with 10 $\mu$l/ml -mercaptoethanol, homogenized (Omni tissue homogenizer, Omni/Tech Quest, Warrenton, VA) at 15,000 rpm for 40 s, and placed on ice ...

*Preparation of total RNA from individual tubules ...*
*Quantitative real-time RT-PCR ...*
*Antibody ...*
*Tissue preparation for light microscopy ...*
*Colocalization of pendrin and TSC immunoreactivity ...*
*Tissue processing for immunoelectron microscopy ...*
*Immunogold labeling ...*
*Electron microscopy ...*
*Morphometric analysis ...*
*Statistical analysis*. For the RT-PCR, data comparisons among three or more groups were made by using ANOVA with Tukey's posttest. For comparisons of gold label density, repeated-measures ANOVA with Tukey's posttest was used. Statistical significance was achieved with a $P < 0.05$. Data are displayed as means ± SE.

---

This was a very complex set of methods and the authors had every intention of ensuring that they could be reproduced. Note again the organization by type of method, how materials (and what sorts of materials) and their sources were described. Take note of the style. The third person, passive voice should be used very sparingly in most parts of a paper, but it is commonly used when describing a methodology. It is, after all, difficult to write methods in active voice without using first person. Pipets don't operate themselves, for example.

**EXAMPLES FROM RESULTS SECTIONS**

### *Item #4* Ontogeny of hypertonic preabsorptive inhibitory control of intake in neonatal rats

Aron Weller, Ludmila Tsitolovskya, Iris H. Gispan, and Gerard P. Smith, *Am J Physiol Regul Integr Comp Physiol 278: R44-R49, 2000*

Figure 1 depicts the effects of the various preloads on P6, presenting the data according to the two levels of deprivation. At this young age control of intake by the volume of the preload was not evident, because the isotonic preload of 0.25 M mannitol did not significantly reduce intake compared with the sham preload [$F(1,51) = 1.69$, $P > 0.198$]. There was a significant effect of deprivation [$F(1,51) = 7.32$, $P < 0.01$] in this analysis, but the preload X deprivation interaction [$F(1,51) = 0.01$, $P > 0.94$] was not significant. Overall, the pups deprived for 24 h ate significantly more than those deprived for 6 h.

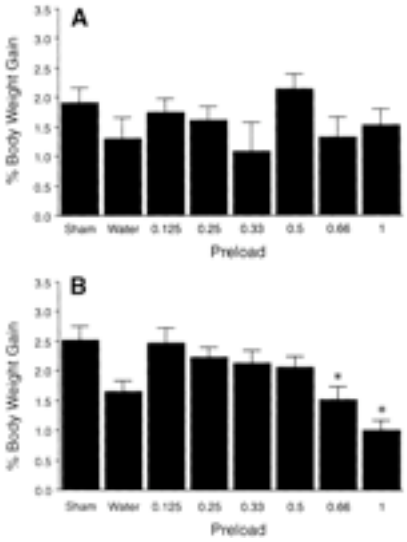


Fig. 1. Effects of gastric preloads (5% body wt) administered 5 min before a 30-min meal on intake (expressed as %body wt gain) in 6-day-old rats. A: data after 6 h of deprivation; n = 7-15 for each preload group. B: data after 24 h of deprivation; n = 12-14 for each preload group. Preloads were sham, isotonic (0.25 M mannitol), hypertonic (0.33-1.0 M mannitol), or hypotonic (water or 0.125 M mannitol). The highest concentrations of mannitol (0.66 and 1.0 M) decreased intake significantly compared with isotonic mannitol only after 24 h and not after 6 h of deprivation; 0.25 M mannitol, the isotonic control for volume of the mannitol preload, did not decrease intake significantly compared with sham preload. *Intakes after 0.66 and 1.0 M mannitol were significantly less than intake after 0.25 M mannitol, $P < 0.05$.

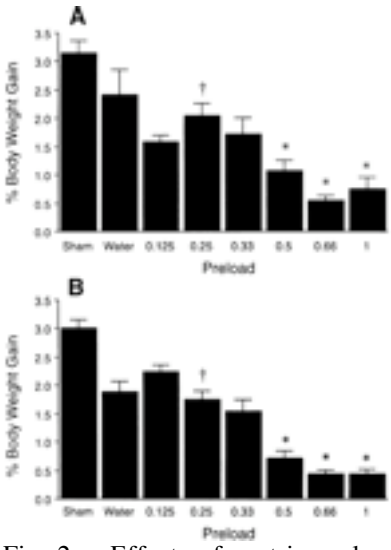


Fig. 2. Effects of gastric preloads (5% body wt) administered 5 min before a 30-min meal on intake (expressed as %body wt gain) in 12-day-old rats. A: data after 6 h of deprivation; n= 19-21 for each preload group. B: data after 24 h of deprivation; n = 11-12 for each preload group. Preloads were sham, isotonic (0.25 M mannitol), hypertonic (0.33-1.0 M mannitol), or hypotonic (water or 0.125 M mannitol). At this age all preloads decreased intake compared with sham preload. Some hypertonic preloads of mannitol (0.50, 0.66, and 1.0 M, but not 0.33 M) reduced intake compared with isotonic mannitol. The hypotonic preloads did not reduce intake compared with isotonic mannitol but did reduce intake compared with sham preload ($P < 0.05$). Intake after preload of 0.25 M mannitol was significantly less than intake after sham preload, $P < 0.05$. *Intakes after 0.5 M, 0.66 M, and 1.0 M mannitol were significantly less than intake after 0.25 M mannitol, $P < 0.05$.

ANOVA using the isotonic and hypertonic concentrations of mannitol yielded a significant interaction [$F(4,121) = 2.71$, $P < 0.05$], in addition to a main effect of preload concentration [$F(4,121) = 3.73$, $P < 0.01$]. The main effect of deprivation was not significant. Post hoc comparisons showed the following pattern of results (see Fig. 3). 1) Intake of the groups that received preloads of isotonic mannitol did not differ over the two levels of deprivation. 2) Among the groups tested after 6 h of deprivation, none was significantly different from the group that received the isotonic mannitol preload. 3) Among the groups tested after 24 h of deprivation, intake was significantly less in the groups that received 1.0 M ($P < 0.01$) and 0.66 M ($P < 0.05$) mannitol compared with the group that received isotonic mannitol. This observation is supported by regression analysis, which showed no significant linear relation after 6 h of deprivation [$R2 = 0.0001$, $F(1,61) = 0.003$, $P > 0.95$] but did show a significant linear regression after the longer level of deprivation [$R2 = 0.30$, $F(1,66) = 28.405$, $P < 0.0001$]. ...CONTINUED

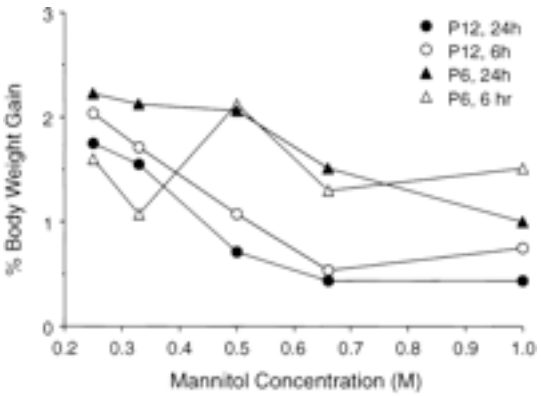


Fig. 3. Inhibitory control of intake by hypertonic preloads of mannitol. Intake (expressed as % body wt gain) is shown as a function of the concentration of the mannitol preload. Data are shown separately for 6- (P6) and 12-day-old rats (P12) after 6 or 24 h of deprivation. The linear regression is significant in all groups except the 6-h deprived, 6-day-old pups. The threshold concentration for intake reduction by mannitol preload (compared with isotonic 0.25 M mannitol) was 0.66 M in 24-h deprived 6-day-old rats and 0.5 M in both groups of 12-day-old rats. Mannitol preload did not affect intake in 6-h deprived, 6-day-old pups.

Note that the results are described in prose, not as figures only. All of the results are described in past tense, since they represent observations by the authors, not generally accepted information. Note the focus on facts. The authors did not try to interpret the data here.

Data with error bars are presented in two different ways. Figures 1 and 2 compared discrete groups of data, thus the bar graph style was appropriate. Figure 3 plotted a continuous dependent variable versus a continuous independent variable. A line graph was most appropriate.

It appears that each individual data point in figure 3 represents a single measurement, thus there are no error bars and the data points are connected. The experimental design (not presented here) required that approach. If the data points were means, then error bars would be needed and curve fits or at least trendlines would be appropriate.

## *Item #9* Identification, Purification, and Characterization of Monoacylglycerol Acyltransferase from Developing Peanut Cotyledons*

A.W. Tumaney, S. Shekar, and R. Rajasekharan , *J. Biol. Chem 276: 10847-10852, 2001*

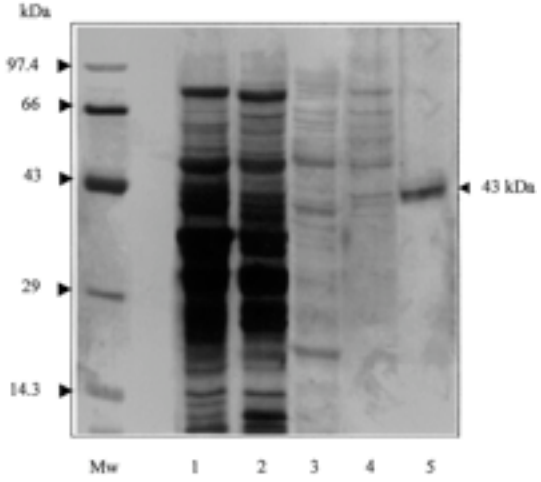


Fig. 4. SDS-polyacrylamide gel electrophoresis profile of MAG acyltransferase purification. Samples from each purification step were separated by 12% SDS-polyacrylamide gel electrophoresis. Lanes 1-5 correspond to the pooled fractions from steps 1-5 (Table I). Lane Mw represents the standard molecular mass marker.

Here is an example of another kind of figure. The figure is neatly and completely labeled, and enough information is included in the caption for the figure to stand on its own.

## *Item #10* Altitudinal variation in parental energy expenditure by white-crowned sparrows

Wesley W. Weathers, Charisse L. Davidson, Christopher R. Olson, Martin L. Morton, Nadav Nur and Thomas R. Famula, *J ExP Biol 205: 2915-2924, 2002*

Table 1. Mean physiological and meteorological variables during doubly labeled water measurements of white-crowned sparrows breeding at Point Reyes Bird Observatory (sea level) and Tioga Pass Meadow (montane) sites

| | Population | | |
|---|---|---|---|
| Variable | Sea level (N=22) | Montane (N=31) | t51 (P)* |
| Body mass (g) | 28.2±1.7 | 28.3±1.7 | 1.03 (0.31) |
| Mass change (%) | 0.02±2.02 | -1.27±1.94 | 2.33 (0.02) |
| Rate of CO2 production (ml CO2 g-1 h-1) | 5.20±0.56 | 6.56±0.86 | 6.49(<0.001) |
| Daily energy expenditure (kJ day-1) | 83.7±9.6 | 103.6±12.2 | 6.33(<0.001) |
| Water efflux (ml H2O kg-1 day-1) | 450±78 | 572±172 | 3.05 (0.004) |
| Standard operative temperature °C) | 11.4±2.9 | 1.1±3.9 | 10.6(<0.001) |

Values are means±S.D.
* Student's t value with probability in parentheses.

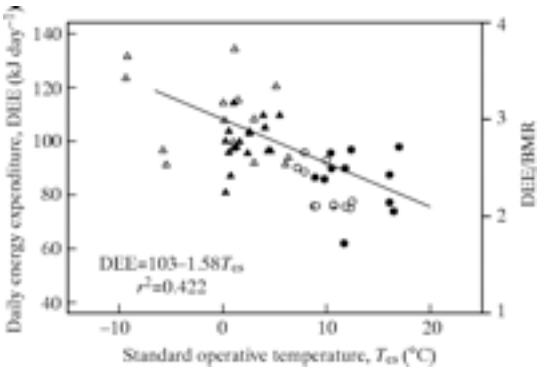


Fig. 3. Relationship between daily energy expenditure and standard operative temperature measured 1 m above ground in the open during the incubation (shaded symbols) and feeding nestling (open symbols) breeding stages for montane (triangles) and sea-level (circles) sparrow populations. BMR, basal metabolic rate.

Here are examples of a table and figure from the same paper (the table has been abridged). The text was extensive and is not reproduced here. We would not simply insert a figure or table in a paper without describing the findings in normal prose in the body of the results.

The designs of tables vary, but some universal rules apply. Use horizontal rules to separate the title from headings, headings from the body of the table (that is, the data), and the body from any footnotes. We don't use vertical rules. As with figures, tables are designed to stand on their own. A reader should not need any additional information in order to know what all of the data represent.

Figure 3 is a scatter plot with two dependent variable axes and carefully selected scales. You won't be doing one of these for the course, but note that despite the differences among bar graphs, line graphs, and scatter plots, all have common features. Note that this graph employed a curve fit using linear regression. The curve fit was kept within the data range, as extrapolation of most data is unwarranted. In this case, there likely are upper and lower temperature limits beyond which the response of the subjects would change qualitatively or they might not even survive.

***Item #11*** **EctoNucleotidase in Cardiac Sympathetic Nerve Endings Modulates ATP-Mediated Feedback of Norepinephrine Release**

Casilde Sesti, M. Johan Broekman , Joan H. F. Drosopoulos , Naziba Islam , Aaron J. Marcus and Roberto Levi , *J Pharmacol Exp The* 300: 605-611, 2002

**Release of Synaptosomal NE by Exogenous ATP and Analogs.** Incubation of cardiac synaptosomes with ATP (0.01-30 $\mu$M for 5 sec) caused a concentration-dependent increase in the release of endogenous NE that reached a maximum of 16% above basal level (EC50 0.96 $\mu$M; Fig. 1). ... [figure one is not reproduced here]

In the presence of the P2XR antagonist PPADS (10 $\mu$M), the NE-releasing effects of ATP, 2-MeSATP, and , $\alpha$,ß-MeATP were all attenuated, as indicated by a marked downward shift in the three concentration-response curves (Fig. 2). ... [figure two is not reproduced here]

In the presence of the P2 receptor antagonist MRS 2179, the NE-releasing effect of ATP was potentiated when MRS 2179 was used at the 30 nM concentration, at which MRS 2179 acts as a selective P2Y1R antagonist (Boyer et al., 1998) (Fig. 3). In contrast, the NE-releasing effect of ATP was attenuated by MRS 2179 at the 30 $\mu$M concentration, at which MRS 2179 acts as a P2XR antagonist (Brown et al., 2000) (Fig. 3).

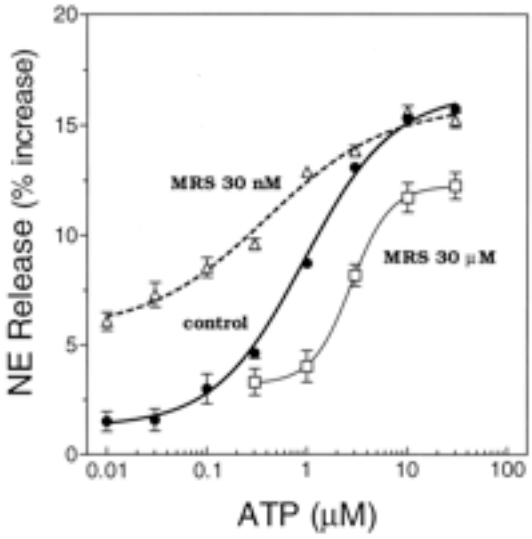


Fig. 3. Release of endogenous NE from guinea pig heart synaptosomes by ATP: modulation by the P2Y1R/P2XR antagonist MRS 2179. Synaptosomes were incubated for 5 s with increasing concentrations of ATP, in the absence (control) or presence of 30 nM or 30 $\mu$M MRS 2179 (n = 8 and 8). Points represent mean increases in NE release above basal level (±S.E.M.). Basal NE level: 1.07 ± 0.16 pmol/mg of protein (n = 16). When not visible, error bars are included in the symbol.

Release of Synaptosomal NE by Exogenous ATP and Analogs: Modulation by the E-NTPDase Inhibitor ARL67156 and by the E-NTPDase solCD39. ... *continued*

At last, someone used error bars and produced what appear to be either trend lines or curve fits. The data were remarkably good, as indicated by "tight" error bars. Note that a couple of data points do not fall on the curve fits, a situation that is expected when data are subject to random (experimental) error.

**EXAMPLES FROM DISCUSSIONS**

## *Item #12* Control of blood pressure mediated by baroreflex changes of heart rate in the chicken embryo (Gallus gallus)

Jordi Altimiras and Dane A. Crossley II , *Am J Physiol Regul Integr Comp Physiol 278: R980-R986, 2000*

The present investigation was undertaken in an effort to establish the critical period at which baroreflexive regulation becomes operational in chicken embryos. In addition, the gain of the reflex was estimated to provide a maturational picture of its development. The data definitely show that the baroreflex is inactive throughout 90% of ontogeny, and it demonstrates a progressive maturation of function in late prenatal and early neonatal periods.

*Critique of method*. The absolute blood pressures reported are based on heart position as determined after freezing. This approach could have resulted in displacement of the embryo during freezing, altering the overall calibration. This is a legitimate concern, considering the heterogenous nature of an egg, with an aqueous albumen phase and a lipid yolk phase. However, the potential error is minimal considering the reduced dimensions of the egg (4 cm as an estimate for the short axis) and the embryonic age used in this study. The maximal error, were zero set at a fixed point along the midline and the embryo situated in the periphery, would be ±0.2 kPa, equivalent to a range of ±20-6% difference in pressure from days 9 to 21. A similar method has also been used in the fetal lamb by adjusting the zero reference to the midline of the uterus (23). Therefore, the reference method proposed here or an alternative should be considered if working with embryos prior to 9 days of age.

An additional criticism could be the use of pharmacological tools to manipulate peripheral resistance in an attempt to invoke the cardiac limb of the reflex. This experimental approach eliminates an important component of the baroreflex and potentially could bias our conclusions. Indeed, several studies in the fetal lamb established that the use of Phe for baroreceptor loading renders higher gains in comparison to the use of vascular occluders (6, 16). It is important to recognize that although sensitivities of the cardiac limb might be overestimated, a reflex was not masked by pharmacological challenge in the fetal lamb, indicating that this approach is valid.

*Comparison with previous studies and between strains*. Blood pressure values reported in this study are in good agreement with previous work on chicken embryos (Fig. 5) and display a progressive rise throughout development. No significant pressure differences were evident

*...continued*

---

The authors began the discussion by restating the objective, then immediately addressed their hypothesis.

Many published research articles are peer-reviewed in order to control the quality of the science. This way we are reasonably certain that most of what we read is scientifically valid. Paragraph two suggests that while the study was deemed acceptable, the reviewers had problems with some of the methods and urged caution in accepting the results without reservations. Students might well adopt this approach to their papers when there is some question as to whether or not an experiment "worked." That is, the data can be accepted, but with some reservations. Skepticism, within reason, is essential, even with regard to one's own data.

### *Item #13* **Short-term insulin treatment and aortic expressions of IGF-1 receptor and VEGF mRNA in diabetic rats**

Tsuneo Kobayashi and Katsuo Kamata , *Am J Physiol Heart Circ Physiol 283: H1761-H1768, 2002*

The main conclusion to be drawn from the present study is that in rats with established STZ-induced diabetes, short-term, high-dose administration of insulin not only normalizes the impaired endothelium-dependent relaxation in the aorta, but also upregulates the expressions of the mRNAs for eNOS and VEGF. Furthermore, these expressions were higher in insulin-treated diabetics than in the controls. These effects in diabetes may be related to the observed increase in the expression of the IGF-1 receptor in the diabetic aorta (insulin untreated and insulin treated).

Our studies are consistent with previous reports (20, 22) on aortas from rats with established STZ-induced diabetes, in which short-term, high-dose administration of insulin normalized the impaired endothelium-dependent relaxation together with an associated increase in expression of the mRNA for NOS and an enhancement of the production of NO. ... Indeed, we found in the present study that the expression of the mRNA for VEGF, which is regulated by IGF-1 (see below; 13, 26, 41), was significantly increased only in insulin-treated diabetic rats...*continued*

... One week of insulin treatment increased the plasma insulin level in both the control and diabetic groups (Table 2), and it also ameliorated endothelial dysfunction and increased eNOS mRNA, suggesting that the hyperinsulinemia present in insulin-treated established STZ-induced diabetic rats may be responsible for these changes.

In conclusion, we found that 1 wk of insulin treatment in diabetic rats led to an enhanced expression of the IGF-1 receptor. This presumably increased the expression of VEGF mRNA, and the increased VEGF presumably upregulated eNOS, thereby resulting in an amelioration in the endothelial dysfunction otherwise seen in diabetic rats. Furthermore, these expressions were higher in insulin-treated diabetic than in control (nondiabetic) rats. On the downside, an increase in the expression of the IGF-1 receptor may be a key event in the progress of atherosclerosis in diabetes. The above effects may follow from the increased IGF-1-receptor expression that occurs in both the insulin-untreated diabetic and insulin-treated diabetic aorta.

---

The authors opened the discussion by presenting the main outcome of the study, an approach that works well. They reported background information from other studies in the context of their own findings, that is, they stayed focused on interpreting their results and comparing them with what is already known.

When discussing the implications of their own findings the authors went into the actual mechanisms that underlie the observations. Their discussion is not simply a recapitulation of the results.

The authors did a nice job of wrapping up their discussion with conclusions.

### *Item #14* **Apoptosis-inducing factor is involved in the regulation of caspase-independent neuronal cell death**

Sean P. Cregan, Andre Fortin, Jason G. MacLaurin, Steven M. Callaghan, Francesco Cecconi, Seong-Woon Yu, Ted M. Dawson, Valina L. Dawson, David S. Park, Guido Kroemer and Ruth S. Slack , *Journal Cell Biol 158: 507-517, 2002*

[finish of the discussion]...The precise mechanism by which Bax mediates mitochondrial membrane permeabilization and the release of apoptogenic factors remains highly controversial. However, two main hypothetical models have been proposed. In the first, Bax is proposed to oligomerize upon insertion into the mitochondria and to directly form pores within the outer membrane. This hypothesis is supported by the finding that Bcl-2 family proteins share some structural homology with the transmembrane domain of diptheria toxin and the colicins, and that Bcl-2 family proteins can form pores in artificial membranes (Muchmore et al., 1996; Antonsson et al., 2000; Saito et al., 2000). In the other model, Bax is proposed to interact with existing membrane channels and to modulate their conductivity. Accordingly, Bax has been reported to interact with the voltage-dependent anion channel and studies in yeast cells and isolated mitochondria have suggested that this interaction is required for Bax-mediated mitochondrial effects (Shimizu et al., 1999, 2000). On the other hand, other research groups have indicated that the inner mitochondrial membrane protein, adenine nucleotide translocator (ANT), is the critical Bax target (Marzo et al., 1998). It is possible that more than one of these models is correct and Bax can form different types of channels. In this case, it is conceivable that the different apoptogenic factors could be released through distinct channels. Alternatively, Bax could alter membrane permeability either by forming pores itself or by modulating existing channels, allowing the selective release of smaller molecules like cytochrome-c. This increase in membrane permeability could lead to swelling of the mitochondrial matrix and eventual lysis of the outer mitochondrial membrane (Vander Heiden et al., 1997), resulting in the release of larger apoptogenic molecules like AIF.

In summary, we have shown that p53 induces neuronal cell death through a caspase-mediated process in the presence of Apaf1, and through a caspase-independent process in the absence of Apaf1. Furthermore, we have shown that AIF is an important regulator of the caspase-independent cell death pathway and functions downstream of Bax. The fact that blocking AIF function with neutralizing antibodies provides significant protection against cell death suggests that AIF may represent an important therapeutic target for neuroprotection after acute injury.

Here is the ubiquitous first person, but they did use it sparingly. Note the focus on mechanisms. A meaningful interpretation requires some depth of understanding.

**EXAMPLES OF ABSTRACTS**

## Item#15 Role of calcium in metabolic signaling between cardiac sarcoplasmic reticulum and mitochondria in vitro

Robert S. Balaban, Salil Bose, Stephanie A. French, and Paul R. Territo , *Am J Physiol Cell Physiol 284: C285-C293, 2003*

The role of $Ca^{2+}$ as a cytosolic signaling molecule between porcine cardiac sarcoplasmic reticulum (SR) ATPase and mitochondrial ATP production was evaluated in vitro. The $Ca^{2+}$ sensitivity of these processes was determined individually and in a reconstituted system with SR and mitochondria in a 0.5:1 protein-to-cytochrome *aa3* ratio. The half-maximal concentration ($K_{1/2}$) of SR ATPase was 335 nM $Ca^{2+}$. The ATP synthesis dependence was similar with a $K_{1/2}$ of 243 nM for dehydrogenases and 114 nM for overall ATP production. In the reconstituted system, $Ca^{2+}$ increased thapsigargin-sensitive ATP production (maximum ~5-fold) with minimal changes in mitochondrial reduced nicotinamide adenine dinucleotide (NADH). NADH concentration

remained stable despite graded increases in NADH turnover induced over a wide range of $Ca^{2+}$ concentrations (0 to ~500 nM). These data are consistent with a balanced activation of SR ATPase and mitochondrial ATP synthesis by $Ca^{2+}$ that contributes to a homeostasis of energy metabolism metabolites. It is suggested that this balanced activation by cytosolic $Ca^{2+}$ is partially responsible for the minimal alteration in energy metabolism intermediates that occurs with changes in cardiac workload *in vivo*.

There are many positive features to this abstract. It is written in one paragraph, it is concise, and there is minimal background information. The significance of the study is implicit in the first sentence, which states the objective. All abbreviations are explained the first time they are used, and there is no reference to other work, tables, figures, etc., that is, the abstract can stand on its own. There is a complete summary of results including relevant quantitative data. The authors referred to the work itself and any specific results in past tense, reserving present tense for established facts or generalizations. The last sentence sums up conclusions that can be drawn from the study.

### *Item #16* Transplacental Transfer and Metabolism of Buprenorphine

Tatiana Nanovskaya, Sujal Deshmukh, Monica Brooks and Mahmoud S. Ahmed , *J Pharmacol Exp Ther 300(1): 26-33, 2002*

Information on the direct and indirect effects of buprenorphine (BUP) on the fetus is essential for determining its potential for treatment of the pregnant opiate addict. The goal of this investigation is to determine the transplacental transfer of BUP to the fetal circulation, its metabolism, and effects on the tissue. The technique of dual perfusion of placental lobule is used. The range of BUP concentrations investigated included its peak plasma levels (10 ng/ml) in patients under treatment. A biphasic decline in concentration of the drug in the maternal circulation was observed, initially rapid then slow. During the initial (60 min), the tissue sequestered most of BUP resulting in a low (<10%) transplacental transfer of the drug to the fetal circulation. The concentration ratios of the drug in tissue/maternal and tissue/fetal were 13 ± 6.5 and 27.4 ± 0.4. The drug sequestered did not have any adverse effects on placental tissue viability and functional parameters. Less than 5% of the perfused BUP was metabolized to norbuprenorphine during the 4 h of perfusion and the metabolite was distributed between the tissue, maternal, and fetal circulations. Taken together, these data suggest that the therapeutic levels of BUP in the maternal circulation may have no indirect effects (via the placenta) on the fetus. The observed low transplacental transfer of BUP to the fetal circuit may explain the moderate/absence of neonatal withdrawal in the limited number of reports on mothers treated with the drug during pregnancy.

The data reported in the gray area are without units, however since the authors reported a ratio, units would cancel out. The significance of a concentration ratio would be obvious to pharmacologists, who comprise the major readership. The method for reporting standard error is not described. The use of the standard deviation to describe experimental error in text is so common that when there is no indication one can assume that the standard deviation was used.

The authors reported one ratio with two significant digits and the other with three, an inconsistency that should have been caught.

### *Item #17* Identification and preliminary characterization of an O6-methylguanine DNA repair methyltransferase in the yeast *Saccharomyces cerevisiae*

M Sassanfar and L Samson , *J. Biol. Chem., 265: 20-25, 1990*

*Saccharomyces cerevisiae* contains a DNA repair methyltransferase (MTase) that repairs O6-methylguanine. Methyl groups are irreversibly transferred from O6-methylguanine in DNA to a 25-kilodalton protein in S. cerevisiae cell extracts, and methyl transfer is accompanied by the formation of S-methylcysteine. The yeast MTase is expressed at approximately 150 molecules/cell in exponentially growing yeast cultures but is not detectable in stationary phase cells. Unlike mammalian and bacterial MTases, the yeast MTase is very temperature- sensitive, having a half-life of about 4 min at 37 degrees C, which may explain why others have failed to detect it. Like other DNA repair MTases, the *S. cerevisiae* MTase repairs O6-methylguanine more efficiently in double-stranded DNA than in single-stranded DNA. Synthesis of the yeast DNA MTase is apparently not inducible by sublethal exposures to alkylating agent, but rather MTase activity is depleted in cells exposed to low doses of alkylating agent. Judging from its molecular weight and substrate specificity, the yeast DNA MTase is more closely related to mammalian MTases than to *Escherichia coli* MTases.

This abstract focused on conclusions and did not include much in the way of specific (quantitative) data. Perhaps the results were best described in qualitative terms, as is the case for many studies in molecular biology.

Because the authors stated their results in present tense it is impossible to determine which of the statements made in this abstract were from prior studies (generally accepted knowledge) and which statements report the authors' actual findings. In science, no finding is generally accepted until it is confirmed independently.

**Item #18 Amyloidogenic processing of the Alzheimer ß-amyloid precursor protein depends on lipid rafts**

Robert Ehehalt, Patrick Keller, Christian Haass, Christoph Thiele and Kai Simons
*Journal Cell Biol 160: 113-123, 2003*

Formation of senile plaques containing the ß-amyloid peptide (Aß) derived from the amyloid precursor protein (APP) is an invariant feature of Alzheimer's disease (AD). APP is cleaved either by ß-secretase or by -secretase to initiate amyloidogenic (release of Aß) or nonamyloidogenic processing of APP, respectively. A key to understanding AD is to unravel how access of these enzymes to APP is regulated. Here, we demonstrate that lipid rafts are critically involved in regulating Aß generation. Reducing cholesterol levels in N2a cells decreased Aß production. APP and the ß-site APP cleavage enzyme (BACE1) could be induced to copatch at the plasma membrane upon cross-linking with antibodies and to segregate away from nonraft markers. Antibody cross-linking dramatically increased production of Aß in a cholesterol-dependent manner. Aß generation was dependent on endocytosis and was reduced after expression of the dynamin mutant K44A and the Rab5 GTPase-activating protein, RN-tre. This inhibition could be overcome by antibody cross-linking. These observations suggest the existence of two APP pools. Although APP inside raft clusters seems to be cleaved by ß-secretase, APP outside rafts undergoes cleavage by -secretase. Thus, access of α- and ß-secretase to APP, and therefore Aß generation, may be determined by dynamic interactions of APP with lipid rafts.

The use of first person is becoming more and more widely accepted — see McMillan pages 145 - 147 about passive and active voice, and the use of first person. However, also take a look at page 65 under Materials and Methods. First person is appropriate under some circumstances, such as when writing an autobiography or when it is important to point out that the writer was the first to make the observations being reported. <u>However the use of first person really does divert the reader's attention to the author and away from the subject itself. The recommendation here is to use both passive voice and first person sparingly.</u>

**EXAMPLES OF INTRODUCTIONS**

A primary purpose of a typical introduction is to provide a context for a study and to defend its objectives and the experimental approach. What was the question or objective and why was it important? How were the objectives accomplished and why was the study done this way? An introduction need not and often should not report results.

### *Item #1* Amyloidogenic processing of the Alzheimer ß-amyloid precursor protein depends on lipid rafts

Robert Ehehalt, Patrick Keller, Christian Haass, Christoph Thiele and Kai Simons
*Journal Cell Biol 160: 113-123, 2003*

Formation of senile plaques composed of a 4-kD small peptide, the amyloid ß-peptide (Aß)* is one of the hallmarks of Alzheimer's disease (AD). Aß derives from a large type I transmembrane protein, the amyloid precursor protein (APP) (for review see Selkoe, 2001). It is cleaved out sequentially by enzymes termed ß- and γ-secretase. ... Since α- and ß-cleavages directly compete for their substrate APP, the key in understanding Aß generation is to find out how access of these enzymes to APP is regulated.

There is growing evidence that cholesterol is of particular importance in regulating α- and ß-cleavage. ... Interestingly, two independent retrospective studies reported a strong decrease in the incidence of AD and dementia in patients treated with 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (Jick et al., 2000; Wolozin et al., 2000).

All of these studies point out that cholesterol is critically involved in Aß generation. However, little is known about the mechanisms by which cholesterol affects this process. ... This model of membrane compartmentalization would explain how the same protein could be processed in two different mutually exclusive ways.

If cleavage of APP by BACE1 occurred in rafts, it would be important to know how and where this interaction is regulated. Therefore, in this paper we have studied these relationships and provide evidence that Aß generation critically depends on lipid rafts for enzyme activation to occur.

---

*The authors included an extensive list of abbreviations and their definitions, not reproduced here.

The authors introduced the significance of the study in the first sentence, then provided relevant background in order to defend their study, ending the first paragraph with a statement explaining the general objective that this specific study addressed. The next two paragraphs further defended the study, providing a context for the specific work conducted by these investigators. Students will not be expected to delve so deeply into the background behind their studies, in fact brevity is often a virtue. Compare this introduction with the one for the complete paper that you have been provided.

The last paragraph states the specific objective of the current study. The authors did not specify what sorts of experiments were conducted, which would have been useful, nor did they describe or defend the experimental model.

The gray area points out the use of a superlative (the word "interestingly"). Such subjectivity, used very sparingly, can be okay. Its use here was to distinguish the result as a major finding with great potential. It is best to avoid subjectivity and superlatives for the most part, though.

***Item #2*** **Maturation and Specificity of Plasmodium falciparum Subtilisin-like Protease-1, a Malaria Merozoite Subtilisin-like Serine Protease**

Mohammed Sajid§, Chrislaine Withers-Martinez¶, and Michael J. Blackman
*J Biol Chem 275: 631-641, 2000*

Malaria is caused by parasitic protozoa of the genus *Plasmodium*. The clinical disease and associated pathology is a direct result of replication of the parasite within host red blood cells. Invasion of red blood cells by the malaria merozoite is prevented by certain serine and cysteine protease inhibitors (1, 2), and merozoite proteases appear to play a crucial role in invasion by restructuring the erythrocyte surface or cytoskeleton (3-5). In addition, a number of merozoite surface proteins undergo extensive proteolytic modification around the point of erythrocyte invasion (6, 7). The best characterized example of this is that of merozoite surface protein-1 an abundant, stably expressed surface protein that is subjected to proteolytic processing and shedding during invasion. ... A full understanding of the mode of activation, structure, and physiological function of PfSUB-1 is essential to evaluate its potential as a therapeutic target.

Subtilases are synthesized as enzymatically inactive zymogens, activation of which invariably requires one or more proteolytic cleavages of the precursor (13). We have previously shown that the primary pfsub-1 gene product undergoes two major intracellular posttranslational processing steps. The first of these, conversion of the earliest detectable 82-kDa translation product to a 54-kDa form (called p54), takes place rapidly following translation and, by analogy with other systems, was proposed to represent an autocatalytic processing step possibly triggered by signal peptide cleavage and co-translational folding within the lumen of the parasite endoplasmic reticulum (ER). ... To understand the requirements for expression of proteolytically active PfSUB-1, we set out to further characterize these posttranslational modifications.

Here, we present the results of a detailed study of the fate of PfSUB-1 as it traverses the secretory pathway of the malaria parasite. Using an in vitro translation-based model, analysis of posttranslational processing of PfSUB-1 in the parasite, and studies of enzymatically active recombinant PfSUB-1, we show that the proteolytic processing to which PfSUB-1 is subjected exhibits characteristics of subtilase zymogen activation, although with some novel features, and results in the production of an enzymatically active subtilase with an unusual substrate specificity.

---

Note how, in paragraph 1, the authors introduced the general subject and its significance, then provided the background necessary for the reader to appreciate why a study of PfSUB-1 would be of value. Paragraph 2 provides the rationale for the experiments that they conducted. Paragraph three focuses on the study - how it was conducted and what the study found.

Note that the model used in the experiment was not defended, which is the norm for scientists who write for a specialized readership. Student-authored introductions should probably provide the rationale for using a particular biological model for a particular study.

It is rather difficult to get away from the use of first person these days. The practice isn't really all that bad, although it helps to reinforce the notion that scientists have large egos.

### *Item #3* **Oxytocin-induced renin secretion in conscious rats**

Wan Huang1, Mats Sjöquist2, Ole Skott3, Edward M. Stricker1, and Alan F. Sved1
*Am J Physiol Regul Integr Comp Physiol 278: R226-R230, 2000*

IN ADDITION to the well-known actions of oxytocin (OT) during lactation and parturition, OT is a natriuretic hormone (25). Indeed, neurohypophysial OT secreted in response to osmotic stimulation in rats has been documented to contribute importantly to the natriuresis observed under these conditions (5, 7). OT is also secreted in large amounts in response to hypotension (14) or hypovolemia (20), although its actions under these conditions remain obscure.

Binding sites for OT exist in the macula densa (19). The macula densa is known to stimulate renin secretion (18), which contributes importantly to cardiovascular homeostasis during hypotension or hypovolemia. In recent studies we noted that intravenous infusion of OT in physiological doses stimulates renin secretion in anesthetized rats and that the action of OT on renin release is not secondary to its natriuretic effects (17). The present studies sought to determine whether infusion of OT increases plasma renin levels in conscious rats. Because the results indicated that infusion of OT did increase plasma renin levels, additional studies were conducted to determine whether this response required -adrenoceptor-dependent mechanisms, which would suggest an action independent of the macula densa.

---

Brevity can be a virtue. Your papers will probably require something a little more extensive, in order to make the necessary points, but perhaps not as extensive as the previous two examples.

Different journals have different peculiarities. Evidently this journal starts the first sentence of its introduction in all capital letters. Why? Your guess is as good as mine.

### *Item #4* **Control of blood pressure mediated by baroreflex changes of heart rate in the chicken embryo (Gallus gallus)**

Jordi Altimiras and Dane A. Crossley II
*Am J Physiol Regul Integr Comp Physiol 278: R980-R986, 2000*

PRESSURE DIFFERENCES between the arterial and venous sides of the cardiovascular system provide the necessary driving force to ensure adequate gas transfer to tissues. Alterations in arterial pressure that may compromise gas transfer are counteracted by both central and local homeostatic mechanisms. These mechanisms offset the perturbation, thereby avoiding hypertensive or hypotensive episodes and the myriad of pathologies these conditions could induce (28).

Among regulatory mechanisms, the baroreflex is the most prominent short-term compensator during arterial pressure challenges. This compensatory system results in an alteration of cardiac output and peripheral resistance via the cardiac and peripheral limb of the baroreflex, respectively. Although baroreflex responses are well characterized in a variety of adult vertebrates (1), little is known of the maturation of this mechanism.

In the fetal sheep, the standard model in mammalian studies of cardiovascular physiology, an unequivocal baroreflex response is present during the final trimester of gestation (6, 16, 22, 23).

Although this model is attractive for its link to clinical pediatrics, an alternative experimental model with short extrauterine development would be instrumental in exploring the course of baroreflex maturation at different organizational levels. Such a model would also be important to the construction of a generalized picture of the ontogeny of cardiovascular regulation in vertebrates.

The chicken embryo may be such a model, with the advantage of a shortened gestational time, ease of embryonic manipulation, and a mammalian-like circulation with an extraembryonic circuit involved in gas exchange (the chorioallantois) analogous to the placenta (17). Furthermore, its use in the analysis of chemoreflexive cardiovascular regulation has recently been shown (18).

Finally, the maturation of many physiological processes is well characterized in chicken embryos, providing essential information for an in-depth study. Previous studies on autonomic cardiovascular regulation indicate that functional vagal innervation appears on day 12 of development (19), implying that a hypertensive baroreflex could be operational during the later half of chicken ontogeny. The intention of this work was to test the hypothesis that baroreflex function appears during incubation and follows a progressive maturation as it occurs in the fetal lamb.

---

This introduction focused a fair amount of attention on the model system itself and why it was appropriate.

The highlighted area is a statement of a specific hypothesis to be tested as the objective of the study. At the risk of being inconsistent, first person would have saved some space and changed passive to active voice. "We tested the hypothesis that..." is more succinct and direct.