UNIVERSITY OF KUOPIO

Juhani Jänne

A Guide to Scientific Writing

Preparing an article and a grant proposal in the biosciences

AIVI Academic Press

A Guide to Scientific Writing

Preparing an article and a grant proposal in the biosciences

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Table of contents

Preface 5
1. History of the scientific article 6
2. IMRAD format as the backbone of a scientific paper 6
3. Writing as a part of the internal scientific process 7
4. Writing a scientific paper 8
4.1. Preparatory work before you start writing 8
4.2. Title and authors 9
4.3. Summary (Abstract, Synopsis) 13
4.4. Introduction (Background) 14
4.5. Materials and Methods (Experimental Procedures) 17
4.6. Results 19
4.7. Discussion (and conclusions) 23
4.8. References 26
5. Writing a review article or book chapter 27
6. Doctoral thesis 28
7. Poster 29
8. Verbal presentation 29
9. Submission of the manuscript and editorial correspondence 30
9.1. Publication forum 30
9.2. The Editorial Offices of scientific journals 31
9.3. Covering letter (cover letter) 32
9.4. Criteria for acceptance 33
9.5. Editorial response 34
9.6. Response to critique 36
9.7. Electronic submission 37
9.8. Publication or patent? 38
10. Linguistic pitfalls and useful phrases 38
10.1. Misused words 38
10.2. Singular/plural and numbers 39
10.3. Nouns as adjectives/adjectives as nouns 39
10.4. Abbreviations 40

10.5. British versus American English 40
10.6. Greek alphabets 41
10.7. Useful phrases 41
11. Tips for statistical analyses 43
11.1. Before statistical analyses 43
11.2 Variability: Standard deviation or standard error of the mean? 44
11.3. Outlier (abnormal value) 44
11.4. Comparing two groups: Parametric tests: *t* test 45
11.5. Nonparametric tests: Mann-Whitney test 47
11.6. Nonparametric tests: Wilcoxon test 48
11.7. Comparing three or more groups: One-way analysis of variance (ANOVA) 49
11.8. Post hoc tests for ANOVA 49
11.9. Nonparametric tests: Kruskal-Wallis test 50
11.10. Nonparametric tests: Friedman test 50
11.11. Two-way ANOVA 51
11.12. Linear regression 52
11.13. Contingency tables 53
11.14 Survival curves (Kaplan-Meier) 54
11.15. Choosing the statistical analysis 55
12. Writing a grant proposal 56
12.1. Organization of the research plan 56
12.2. Title 57
12.3. Abstract (Summary) 57
12.4. Background and significance 58
12.5. Objectives, approaches and methods 58
12.6. Research group and resources 59
12.7. Results 59
12.8. Budget and budget justification 60
12.9. References 60
12.10. Curriculum vitae 60
12.11. Evaluation of a grant application 62
12.12. Personal grant 63
12.13. Scientific writing and the society 63
13. The ethics of research 64

13.1. Violations of good scientific practice 65
13.2. Scientific fraud 66
13.3. Procedures in suspected scientific fraud 67
14. Further readings 68

Preface

This book is aimed to be a guide of scientific writing for researchers and graduate students in the biomedical sciences. The approach of the book is strictly practical as it follows an authentic example article section by section focusing on the special organization of each part of the paper including the appropriate use of the tenses of verbs. It likewise contains words and phrases, which may create problem at least to those not native in the English language. The book also guides readers to the secrets of editorial correspondence and editorial processes and contains tips on statistical analyses in the form of simple examples. The last parts of the book deal with the preparation of a successful grant proposal and the ethics of the research.

The book is mainly aimed at graduate students preparing their first scientific papers but it may be useful also for more experienced scientists, who may, to their surprise, find that scientific writing includes distinct rules and technical formalities.

The book is mainly based on the lectures of the author given over the past several years at the University of Kuopio. It is the impression of the author that there is a need for this kind of practical guide.

I thank Professors Leena Alhonen and Garry Wong for their extremely helpful comments. Garry Wong also kindly revised the English language. The *American Society for Biochemistry and Molecular Biology* kindly granted permission to use the example article in the book.

Kuopio, 2005 Juhani Jänne

1. History of the scientific article

The publication of the first scientific series (*Philosophical Transactions*, London) started already more than 300 years ago. The first publications only remotely resembled the present-day scientific articles being informal and descriptive, sort of "case reports". The modern scientific paper started to develop at the turn of the 20th century when distinct rules were introduced to define the structure and organization of different parts of the article. This was the emergence of the so-called IMRAD format that is exclusively used in all current scientific journals.

2. IMRAD format as the backbone of a scientific paper

IMRAD format (the abbreviation stands for I, *Introduction*; M, *Methods*; R, *Results*; *And* D, *Discussion*) has been developed during the last 100 years and it is today exclusively used by all scientific journals. Even though the recommendation to use the IMRAD format in scientific writing was introduced already at the turn of the 20th century, the scientific journals only slowly adopted the format in the 1970´s when about 80 % of articles in medical journals adhered to the IMRAD format. Apparently, the basic science journals adopted the format much earlier, in the late 50´s and 60´s. A few current journals follow a slightly different IRDAM format, in which the Methods are described in the last section of the paper.

IMRAD logic:

- **What was the problem studied? Answer = Introduction**
- **How was it studied? Answer = Methods**
- **What were the results? Answer = Results**
- **And**
- **What do the results mean? Answer = Discussion**

The IMRAD format established fixed rules for scientific writing even determining the tense of the verbs (past or present) in a given section of the paper. The format not only gives rules for the writing, it also makes the reading of the paper easier as given items can be found in particular sections. The format likewise facilitates the peer re-

view process. Although the format lacks the acronym for *Abstract*, also the abstract itself is structured according to IMRAD. The use of the format is not confined to primary publications as it is also used in posters, verbal presentations and, to some extent, even in medical case reports.

3. Writing as a part of the internal scientific process

An individual scientist and scientific article belong to a network where journal editors and independent reviewers act as major players. At best, the scientific publication is included in textbooks after being assessed by the scientific community (Fig. 1).

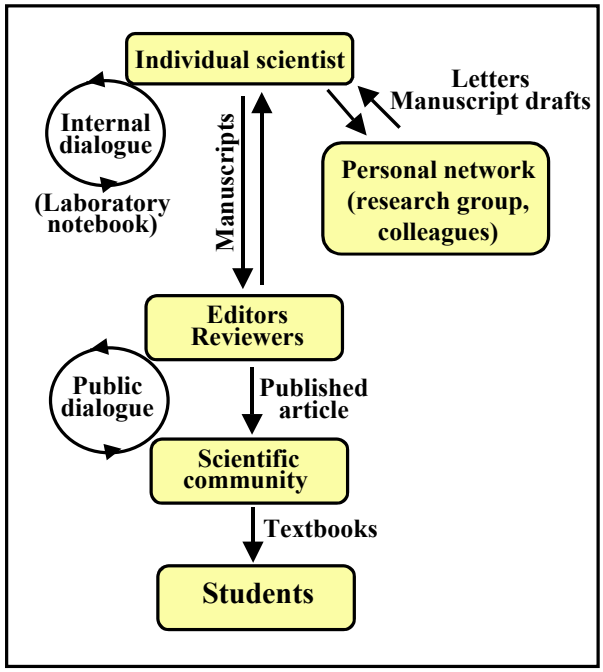

Fig. 1. *Individual scientist and the internal scientific process*

How important is publishing? The unambiguous answer is: Research, independent of how revolutionary it is, is nothing until published. Robert A. Day compares in his book (see chapter 14) scientific research with the fall of a tree in the forest: "*If a tree falls in the forest and there is no one to hear it falling, does it make a sound? The correct answer is no. Sound is more than pressure waves, and indeed there will be no sound without a hearer.*" The saying "*Publish or perish*" most accurately describes the importance of publishing in our current scientific community.

4. Writing a scientific paper

Good scientific writing probably requires more organizational than literary skills, yet the latter gift certainly is not a disadvantage.

4.1. Preparatory work before you start writing

Careful preparation for the final writing makes the whole process a lot easier. The preparatory work includes the collection and organization of the experimental material, such as the composition of tables and design of the layout of the figures. At this stage, the data have been analyzed and the statistical analyses have been carried out. The illustrative material (tables and figures) is arranged according to their order of presentation. There may be tables, the content of which is presented in the text (e.g. negative results) instead of formal tables. Fig. 2 depicts an example of the outlining of the structure and content of the paper before writing.

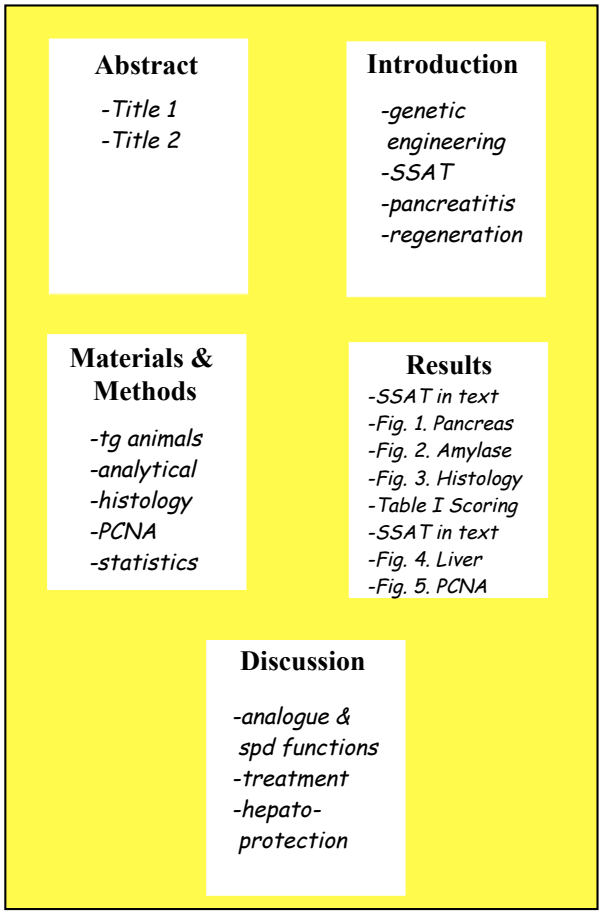

Fig. 2. *Outlining of the structure and content of a paper before writing.*

It is of paramount importance to become familiar with the instructions to authors of the selected journal and to carefully follow them (appearance of the references in the text, the style of headings and subheadings, the style of figure legends and table captions etc). A copy of a recent article published in the selected journal greatly helps to properly organize the paper. The instructions should be followed from the very first draft. Those who are not native in English language are strongly advised to write in English (see also the differences between British and American English) from the beginning and not to translate the manuscript afterwards. At this stage, things, such as the title (Fig.2) may change.

When the writing is outlined, a descriptive name in the form of a single sentence may be given to each figure and table. By reading these few sentences, the main message of the manuscript should become evident. Even a scientist thinks narratively. Science is a story to be told. Give your paper a narrative structure that links one finding to another and describes why certain experiments were performed in response to the results of another experiment.

4.2. Title and authors

The title should describe the main content of the paper as well as possible and should not to be too general or specific. The title should likewise be attractive as thousands read the title, a few, possibly no one, the whole article. The title is not a sentence and hence the verb is often unnecessary. It is advisable to get familiar with the title styles of the selected journal. Some journals apparently dislike “categorical” titles, such as “The cause of X is Y”. Some journals do not like hanging titles. One disadvantage of the hanging titles is also the fact that the last part of the tile may be lost upon citation. The title should be prepared already before the start of writing as it influences the presentation of the data. A short title (running title) with a defined maximum number of characters (usually 60) is often required and should be prepared already at this stage as it should condense the essential message of the title. Most of the journals require 5 to 6 key words that do not appear in the title. Some journals require the key words in so-called MeSH (*Medical Subject Headings*) format. These can be easily obtained from the PubMed database; type your keywords for search and click “*details*” to get the words in MeSH format. One should be careful with the syntax of the title. There are a vast number of examples of titles in the literature, which are rather humoristic due to syntax errors. The following title with faulty order of words

actually means that virus has created mice: "*Mechanism of suppression of pneumonia in mice induced by virus.*" It should read: "*Mechanism of suppression of pneumonia induced in mice by virus.*" The use of dangling participles entirely changes the meaning of the following title indicating that bacteria cause mastitis by gas-liquid chromatography: "*Characterization of bacteria causing mastitis by gas-liquid chromatography.*"

There are no widely accepted rules to determine, who is entitled to be listed as an author. Every author should have a distinct contribution to the planning and execution of the experiments or writing or critically revising the manuscript. The following guideline updated in 2001 (http://www.icmje.org) by the so-called Vancouver Group (*International Committee of Medical Journal Editors*) covers very well the requirements for authorship.

Authorship credit should be based only on (1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

Conditions 1, 2, and 3 must all be met.

Acquisition of funding, the collection of data, or general supervision of the research group, by themselves, do not justify authorship.

The last sentence of the guideline is interesting as in implies that acquisition of funding, collection of data or supervision of the research group do not automatically justify authorship. This part of the guideline is probably most often violated upon preparation of the list of authors. It should also be noted that every author has to approve the final version of the manuscript.

The order of the names in the list of authors sometimes creates problems. It is widely accepted (at least in biomedical journals) that the first author is a person whose contribution to the experiments has been most crucial. The case that two authors have had identical contribution can be shown: Author A* and author B* in which * refers to footnote *"*equal contribution.*" The last author usually is the leader of the research group proving that he/she has essentially contributed to the experiments or preparation of the manuscript. According to international practice, the list of authors is read

into both directions: the first authors represent junior scientists and the last authors senior scientists. In other words, the most important authors of the publication are the first and last one. In most cases, the use of common sense is in order. This is exemplified by the following instance of the relationship between researcher and technician. The researcher plans the experiments and the technician carries them out. Everything goes as planned: the researcher is the single author and the technician is thanked under the acknowledgements. In another case, the outcome of the experiments is not as planned but the technician proposes changes in the experimental conditions whereafter everything works out: now the researcher is the first author and the technician is the second author. In many cases, an experienced technician can work as independently as a researcher and can thus be included as an author, but usually not as the first or last one.

When the manuscript is sent for publication, one of the authors has to act as corresponding author who communicates with the editorial office of the journal. The corresponding author responds to editorial queries and provides additional information as requested. In many cases, the corresponding author can sign the copyright transfer for all authors, yet some journals require that each individual author must sign the transfer. The contact information of the corresponding author appears in the title page of the manuscript, the form of which is defined in the instructions to authors of the journal. Enclosed is an example of the title page.

A polyamine analogue prevents acute pancreatitis and restores early liver regeneration in transgenic rats with activated polyamine catabolism*

Tiina-Liisa Räsänen, Leena Alhonen, Riitta Sinervirta, Tuomo Keinänen, Karl-Heinz Herzig, Suvikki Suppola, Alex R. Khomutov[†], Jouko Vepsäläinen[‡], and Juhani Jänne[§]

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Note that the contact information also contains the street address, as courier shipments cannot be delivered at post office boxes. Unlike the example, most journals request the inclusion of a short (running) title and key words in the title page.

Enclosed is an example of the title as it appears in the Journal of Biological Chemistry.

The JOURNAL OF BIOLOGICAL CHEMISTRY VOL 277, No. 42, Issue of October 18, pp. 39867-39872, 2002

A Polyamine Analogue Prevents Acute Pancreatitis and Restores Early Liver Regeneration in Transgenic Rats with Activated Polyamine Catabolism*

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*This work was supported by grants from the Academy of Finland.
The costs of publication pf this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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The title could also be without verb: "*Prevention of acute pancreatitis and restoration of early liver regeneration by a polyamine analogue in transgenic rats with activated polyamine catabolism.*" Unlike most journals, the Journal of Biological Chemistry shows the funding agencies in the footnote of the title page. Most commonly, the grants are listed under the acknowledgments. As in this case, many journals have their own "hierarchy" for symbols. As the journal has a page charge, this is indicated in the footnote. The title page likewise indicates the date when the manuscript was received for publication as well as the date of final acceptance.

4.3. Summary (Abstract, Synopsis)

It is advisable to write the first draft of the summary right after the preparation the title, yet it can be modified after the completion of the whole manuscript. Writing the summary requires a thorough acquaintance with the obtained results and an extraction of the most important message of the work. The summary is not a detailed description of the work done; use words rather than exact numbers. Usually, the summary does not contain references, but if needed, they should be written in full (not numbered as in the main body of the text). Verbs in the Summary are in *past tense* when one´s own new results are described. The overall organization of the summary adheres to the IMRAD format. In other words, it starts with introductory sentences (1 to 2) followed by the description of own results and ending with conclusions or discussion. The verbs in the introduction and conclusion parts of the summary are in the *present tense*. Never close the summary with a phrase like "*Results will be discussed*." Practically all journals set a maximum length for the summary that is usually from 100 to 250 words (the latter is most common). The summary does not contain information or conclusions that are not in the main body of the text. On the other hand, the Summary does not contain every result obtained. Some journals use structured summary with titles: *Background….Methods….Results...Conclusions*. Enclosed is an example of an authentic summary.

Note that the summary is clearly divided into three different parts (shown with ☛). The two first sentences represent the introduction and contain a reference that is written in full. The verbs are in the *present tense*. The introductory sentences are followed by the description of the results and references to the used methods. Here the verbs are in the *past tense*. The last part of he the Summary represents conclusions and/or discussion. Here the verbs are again in the *present tense*.

The example summary contains 215 words that is less than the maximum length (250) defined by the journal. The condensation of the summary to the defined maximum length sometimes looks like an overwhelming task, but it almost always succeeds without compromising the essence of the message.

☛We recently generated a transgenic rat model for acute pancreatitis, which **is** apparently caused by a massive depletion of pancreatic polyamines spermidine and spermine due to inducible activation of their catabolism (Alhonen, L., Parkkinen, J.J., Keinänen, T., Sinervirta. R., Herzig, K.H., and Jänne, J. (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97, 8290-8295). When subjected to partial hepatectomy, these animals **show** a striking activation of polyamine catabolism at 24 h postoperatively with a profound decrease in hepatic spermidine and spermine pools and failure to initiate liver regeneration.
☛Here we show that pancreatitis in this model **could** be totally prevented, as judged by histopathology and plasma □-amylase activity, by administration of 1-methylspermidine, a metabolically stable analogue of spermidine. Similarly, the analogue, given prior to partial hepatectomy, **restored** early liver regeneration, as indicated by a dramatic increase in the number of proliferating cell nuclear antigen-positive hepatocytes from about 1 % to more than 40 % in response to the drug. ☛The present results **suggest** that the extremely high concentration of spermidine in the pancreas, in fact the highest in the mammalian body, **may** have a critical role in maintaining organ integrity. The failure to initiate liver regeneration in the absence of sufficient hepatic polyamine pools similarly **indicates** that polyamines **are** required for proper commencement of the regenerative process.

4.4. Introduction (Background)

The Introduction is not only a presentation of the background of the study but it also aims to show the gaps in existing knowledge that the present work intends to fill. Introduction is also the place where the motive for the work is presented. The Introduction is not an encyclopedic coverage of the entire literature, but it cites relevant original and review articles. The verbs are in the *present tense* when published works are cited. This is actually part of the ethics of science as published studies are considered as scientific facts. Example: "*Inhibitors of polyamine biosynthesis offer a meaningful target for cancer chemotherapy* [6]." Except: "*Marton et al.* [7] *showed that polyamines are important organic cations*." However, these rules should not be followed slavishly, but common sense must be used. Referring to one´s own published works the *present tense* is principally used, yet sometimes it is more natural to use the *past tense*, especially if the sentence begins like: "*We recently generated transgenic rats that were*…." See also the example abstract where the verbs in the introductory sentences might have been in the *past tense* as well.

The Introduction is usually shorter than the Results and Discussion sections (occasionally only 2 to 3 paragraphs).

The verbs (bolded) in the example introduction below are all in the *present tense*, as published studies are cited.

The polyamines spermidine and spermine and their precursor putrescine **are** intimately associated with growth and differentiation of mammalian cells, yet their exact cellular functions have not been solved (1). In attempts to elucidate the physiological roles of the polyamines, we have generated a number of transgenic mouse and rat lines with genetically altered polyamine metabolism. The activation of polyamine biosynthesis through an overexpression of ornithine decarboxylase **brings about** many interesting phenotypic changes, such as male infertility (2,3), yet these studies are complicated by the fact that overexpression of ornithine decarboxylase only **expands** tissue putrescine pools as the diamine **is** not further converted to spermidine and spermine (4,5). Much more severe distortion of tissue polyamine pools has been achieved by activation of polyamine catabolism through an overexpression of spermidine/spermine N^1-acetyltransferase (SSAT)[1] in transgenic rodents. The latter enzyme **catalyzes** the rate-controlling reaction in the catabolism of spermidine and spermine. After being acetylated, spermidine **is** converted to putrescine and spermine to spermidine by the action of polyamine oxidase (6). Overexpression of SSAT in transgenic rodents **results in** profound changes in tissue polyamine pools, such as the massive accumulation of putrescine, appearance of N^1-acetylspermidine, and decrease in spermidine and/or spermine pools (7). The alterations in polyamine homeostasis **are** accompanied by bizarre phenotypic changes, such as the early and permanent loss of hair, extensive wrinkling of the skin upon aging, lack of subcutaneous fat (7), and reduced life span (5). We recently **generated** transgenic rats in which SSAT expression **is** driven by heavy metal-inducible mouse metallothionein I promoter (8).

The first abbreviation (SSAT) of the paper appears in the introduction (there were no abbreviations in the summary, which is advisable). The superscript refers to the list of abbreviations in the footnote of the first page. The term *rate-controlling* (underlined) is infrequently used in comparison with the extremely widely used term *rate-limiting*. Both refer to a reaction of a metabolic pathway, the rate of which is the slowest among the enzymes of the pathway. *Rate-controlling* is the preferable term as a metabolic pathway may function entirely normally even at half maximum rate of the controlling enzyme where the latter reaction is not any more limiting.

The example introduction continues. In articles of most biomedical journals, the last paragraph of the Introduction serves as an additional summary, which is, however, written in a different way and may have a distinct approach in comparison with the ordinary summary. The verbs are again in the *past tense* when one´s own results

> The metallothionein promoter **directs** the expression of SSAT mainly into liver and pancreas in a heavy metal-inducible fashion. Exposure of the transgenic rats to nontoxic does of zinc, **results** in an immense induction of SSAT activity in the pancreas, a profound depletion of pancreatic spermidine, and spermine pools and acute pancreatitis (8). The fact that pancreatitis **can** not be prevented by inhibition of polyamine oxidase, which generates hydrogen peroxide and a reactive aldehyde, **led** us to conclude that the organ inflammation **is** causally related to the profound depletion of spermidine and spermine (8). We subsequently subjected these transgenic rats to partial hepatectomy and found a striking stimulation of SSAT activity that was associated with a rapid depletion of hepatic spermidine pool at 24 h after the operation (9). Under these conditions, the transgenic rats **failed** to initiate liver regeneration, as judged by lack of proliferative activity and organ weight gain. The regeneration **was** restored only after spermidine concentration returned to the preoperative level, presumably due to enhanced ornithine decarboxylase activity (9).
> ☛Using the transgenic rats with activated polyamine catabolism, we show here that zinc-induced pancreatitis **could** be prevented by a prior administration of 1-methylspermidine, a metabolically stable analogue of spermidine that **is** supposed to fulfill most of the putative cellular functions of spermidine. In a similar fashion, the analogue **alleviated** the proliferative block in the transgenic rats, which was in all likelihood **caused** by spermidine depletion during early liver regeneration.
> ☛ These experiments **appear** to indicate that spermidine **is** specifically involved in the maintenance of pancreatic integrity and in the initiation of rat liver regeneration.

are described except in the last sentence, which represents conclusions. In a randomly selected sample of articles in biomedical journals, about 75 % of papers adhered to this style while in the rest of papers the last paragraph was something like the following: ”*We have studied here the relationship between X and Y*.” In terms of the whole article, the conclusion may appear in three different places: In the Summary, in the last paragraph of the Introduction and in the Discussion sections.

Most of the verbs in latter part of the Introduction are in the *present tense* when one´s own published studies are cited, yet in many instances it is a matter of taste whether to use *present* or *past tense*.

The very last paragraph (☛) of the Introduction now represents the summary, the wording of which differs from that in the ordinary Summary and the last sentence (☛) contains conclusions with verbs in the *present tense*.

4.5. Materials and Methods (Experimental Procedures)

This section should give sufficiently detailed information of the methods that allows a competent scientist to repeat the experiments. If the method used has been published in a standard journal, no description is needed and a journal citation is sufficient. However, if the method has been published in an exotic forum (e.g. "*Savolax Journal of Gastrointestinal Diseases of the Mosquito*"), which is not easily available, the description of the method is surely in order. Sometimes when citing published methods, the citations are linked in such a way that the author cites his/her own modification of the method without giving the description of the original method. This may create problems to find out the original method.

Subheadings are commonly used in Material and Methods section. The first subheading covers usually biological materials (patients, animals, cells etc) and the subsequent subheadings cover materials (including chemical syntheses) and analytical methods. The last subheading covers statistical methods. Conventional statistical analyses (*t* test, analysis of variance, etc.) are neither described nor cited. In the case when commercial software has been used, the name of the package and its supplier are given. Be careful with the syntax of the description of the methods. The following is an example of a very agonizing method. "*After standing in boiling water for an hour, we examined the flasks*." The following example represents a "soluble" method. "*The radioactivity was determined by the trichloroacetic acid-soluble method of Britten et al*." In this section all the verbs are in the *past tense*, except: "*The data <u>are</u> expressed as…*".

The Materials and Methods section is usually the easiest part to write and therefore is tempting as a starting point for the writing. Although this is not forbidden, it is highly advisable to start the writing from the Summary.

Even though an article dealing with basic research and a clinical paper are structurally identical, the two types of papers differ from each other particularly with regards to the Materials and Methods section. In a clinical study, this section is usually divided into three parts. The first part is *Study design* describing the method of ran-

domization, type of blinding (single-blind, double-blind, open etc), type of control (placebo, active medication), parallel groups or cross-over and single-center or multi-center. The second part is *Study population*, i.e. healthy subjects or patients with particular disease, inclusion and exclusion criteria, health conditions, age, gender, ethnic background, height and weight, ethics-related issues, such as written informed consent, protocol reviewed and approved by Institutional Review Board. The third part describes the *Treatments*: drugs and dosages, route of administration, composition of placebo (if placebo-controlled). For drugs, generic names are used (after first mention, give the trade names, manufacturer and the location of the manufacturer). These three parts can also be combined under a single subheading of "*Subjects and study design*".

Below is an authentic example of "*Experimental Procedures*".

EXPERIMENTAL PROCEDURES

Generation of Transgenic Rats- The production of transgenic Wistar rats harboring the metallothionein-SSAT fusion gene (10) has been described earlier (8, 11). Partial hepatectomy **was** carried out according to the original method of Higgins and Anderson (12). The Institutional Animal Care and Use Committee of the University of Kuopio and the Provincial Government **approved** the animal experiments.

Chemicals- 1-Methylspermidine **was** synthesized from 3-aminobutanol as described earlier (13) and administered in saline. Zinc **was** administered as zinc sulfate dissolved in distilled water.

Analytical Methods- Polyamines and their derivatives **were** determined with the aid of high-performance liquid chromatography as described by Hyvönen *et al*. (14). SSAT activity **was** assayed according to Bernacki *et al*. (15). □-Amylase activity **was** determined from heparinized plasma using an analyzer system Microlab 200 from Merck.

Histological Analyses of the Pancreatic Specimens- Formalin-fixed pancreatic specimens **were** embedded in paraffin, cut into 5-□m-thick slices, and stained with hematoxylin/eosin. The stained section **were** coded and blindly **scored** by the participating gastroenterologist (Karl-Heinz Herzig) according to the method of Niederau *et al*. (16). The details of the histological scorings **are** presented in Table I.

Immunohistochemistry of Proliferating Cell Nuclear Antigen (PCNA)- PCNA **was** detected from formalin-fixed paraffin-embedded tissue sections as described in detail earlier (9).

Statistical Analysis- The data **are** expressed as means ± S.D. One-way analysis of variance with Dunnett´s *post hoc* test for multiple comparisons **was** used for the statistical analyses with the aid of a software package, GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, CA).

Note that the section contains several subheadings. With two exceptions, all the verbs are in the *past tense*. For understandable reasons, the verbs are in the present tense in expressions: "*Scorings <u>are</u> presented in Table I*" and "*The <u>data</u> are ex-*

pressed as." As the paper includes animal experiments, these must have been approved by the "*Institutional Animal Care and Use Committee (IACUC)*." As shown, there is no citation to and no description of the statistical method (one-way analysis of variance), but the commercial software package and its supplier are given.

4.6. Results

In terms of readability, the text should be intelligible without the illustrative material (figures and tables) and the illustrative material understandable without the text. The verbs are always in the *past tense* when one´s own new results are described, except "*Table 1 shows*" and "*Fig. 1 depicts*"). If the data are presented in a figure, they are not duplicated in a table or *vice versa*. The legends to the figures are on separate sheets with numbering. The figures likewise are on separate sheets. This means that the figures are not embedded in the text, although the journal may require to mark their approximate positions in the manuscript. The tables are also on separate sheets with their titles and captions (mostly as footnotes to the tables). Some journals (short articles) combine the Results and Discussion sections (*Results and Discussion*), but this is less readable than separate sections. Most journals do not encourage the inclusion of discussion in the Results section.

A central question in the presentation of results is whether to use a table or a figure. There is no unambiguous rule, but generally a table can be used always whereas a figure may become unreadable if containing too many variables. If one likes to give exact numerical values then a table is better, but if one likes to show trends of the data, then a figure is more illustrative. If the numbers just sit there then a table is appropriate. Decide also how many significant digits (3 to 4) are used in the tables and be consistent.

Many authors tend to use tables and figures even in cases where the experimental

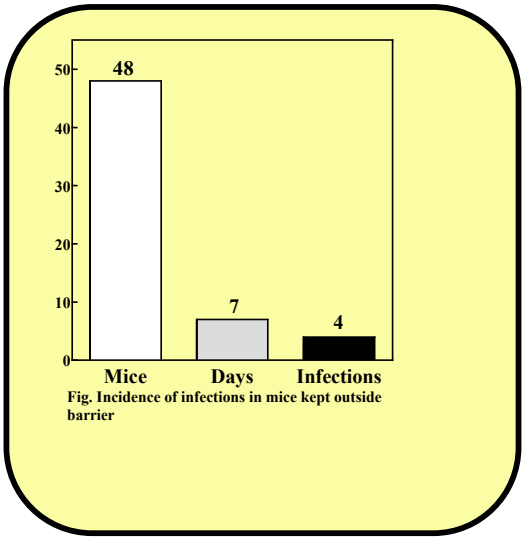
Table 1. Sensitivity of wild-type and transgenic mice to hepatotoxins

Wild type	Transgenic
5/35 (14)[a]	9/34 (26)

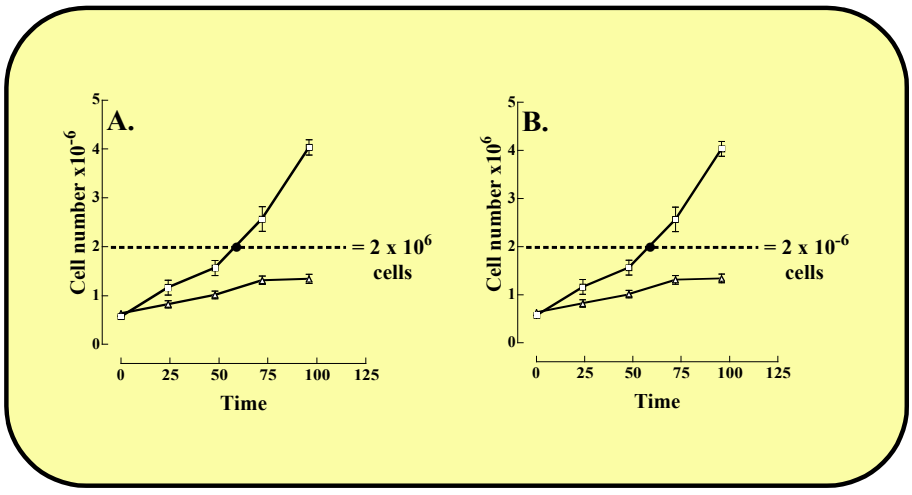
[a]Number of deaths/total (% within parenthesis). p = 0.24.

results could be more appropriately presented in the text, especially when there are only a few variables. The example above represents an entirely unnecessary table. There is no need to tabulate the results, especially as the difference is not statistically significant (p = 0.24). If the findings should have to be included, the following sentence in the text would do it. "*The difference between the mortality rates - 15 % (5/35) for wild-type and 26 % (9/34) for transgenic mice - was not statistically significant*."

Similarly, the figure shown below is unnecessary. It can be stated in the text with a following phrase: "*Among 48 mice, which were transferred out of barrier for an average of seven days, four aquired infection*."



Caution should be exercised when numbering the Y-axis. The following example represents an experiment where cell growth has been recorded as the function of time.



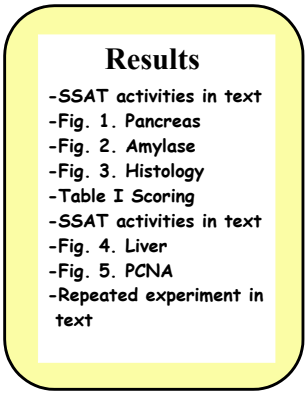
The number of the cells at each time-point is reported as millions and hence the correct exponent will be 10^{-6} (the numbers are divided by one million) and not 10^{6} (the

numbers are multiplied by one million). The confusion in this respect may have been derived from tachometers of the cars where the revolutions/min appear as 1-2 digits and it is indicated that they have been multiplied by one thousand (rpm x 1000).

Some journals limit the total number of figures and tables. The number of the figures can be reduced by combining several figures as different panels in the same figure. The above figure represents one figure with two panels (A and B).

Microscopic pictures should be provided with scale bars, with the aid of which the magnification can always be easily computed (the size of the picture can change during the processing of the manuscript).

Before starting to write the Results section, the order of the presentation of tables and figures can be outlined on separate sheets of paper. According to the sketch below, the first results ("*SSAT activities*") will only be presented in the text (not as a table or figure) followed by figures and tables in their running order. The results of the repeated experiment are incorporated into the text with exact numerical values and statistical significances.



The following authentic parts of the Results section are organized according to the outline above. The section begins with results that have not been tabulated but are incorporated into the body of the text (☛). Note that all the verbs are in the *past tense* when one´s own results are described, except: "*Fig. 1d depicts the accumulation…*"

In the later part of the Results section, the verbs likewise are consistently in the *past tense*, when the present results are described. Exceptions are the sentences describing PCNA (*proliferating cell nuclear antigen*) where the verbs are in the *present tense*: "*PCNA is a convenient and commonly used method to grade proliferative activity in various tissues. PCNA expression is closely correlated with the S-phase of the*

cell cycle (17)." The obvious reason for the use of the *present tense* is the citation to existing literature. In addition: "*Fig. 5 shows*…" and "*Fig 5b depicts*…"

The Results section ends up with the description of the results of the repeated experiment. Principally, every experiment should be repeated in science. In the vast majority of instances, the results of a repeated experiment are settled in the text with the following sentence: "*The experiment was repeated essentially with identical results*."

RESULTS

Depletion of Pancreatic Polyamines by Zinc- ☛ Administration of zinc (10 mg/kg) alone or with methylspermidine (50 mg/kg) **did** not influence SSAT activity in syngenic rats, whereas in transgenic rats, the enzyme activity **rose** from 36 ± 7.1 to 4270 ± 560 pmol/mg/10 min in response to zinc. Inclusion of the analogue with zinc only slightly **increased** SSAT activity over that achieved with zinc alone. ◆ The changes in pancreatic polyamine pools in response to zinc and methylspermidine **are** depicted in Fig. 1. Putrescine pools (Fig. 1a) **remained** very low regardless of the treatment in nontransgenic animals, whereas transgenic animals typically **showed** very high putrescine pools already without any treatments, indicative of constitutive activation of polyamine catabolism. The various treatments only marginally **altered** pancreatic putrescine pools (Fig. 1a). The pancreatic spermidine pool **remained** virtually unaltered after zinc alone or with the combination of the analogue in the syngenic animals (Fig. 1b) but **was** dramatically (by 90%) reduced in transgenic animals in response to zinc. Administration of methylspermidine **had** little effect on zinc-induced depletion of spermidine (Fig. 1b). Although zinc alone or in combination **appeared** to decrease the pancreatic spermine pool in syngenic animals, its effect in transgenic animals **was** much more striking as the spermine pool **was** decreased by more than 80% (Fig. 1c). Fig. 1d **depicts** the accumulation of the analogue in the pancreas after a single dose or two doses. As indicated in the figure, the analogue effectively **accumulated** in the pancreas, apparently with no further metabolism.

On the other hand, if the experiment has been properly repeated and analyzed, the actual results can be presented in the paper as well. Due to possible space limitations, the results can be incorporated into the text instead of using tables or figures. As shown in the later example of the Results section, the numerical results of the experiment have been given with scatters, statistical analyses have been carried out, and the significances between the differences of the means have been indicated.

The very last sentence (◆) of the Results section distinctly represents a conclusion and hence the first verb is in the *present tense*: "*It thus <u>appears</u> that*…" The ex-

amples of the Results section are incomplete, as the pages containing illustrative material have been intentionally omitted.

Effect of Partial Hepatectomy and Methylspermidine on liver Weight Gain and Proliferative Activity- Fig. 5 **shows** liver weight gain (Fig. 5a) and the PCNA labeling index (Fig. 5b) at 24 h after partial hepatectomy without or with a prior injection of methylspermidine in syngenic and transgenic animals. The weight gain of the liver remnant **was** significantly increased in syngenic animals, but not in transgenic animals, at 24 h after the operation (Fig. 5a). Methylspermidine **had** no effect on the weight gain in non-transgenic animals but significantly **increased** the organ weight in transgenic animals (Fig. 5a). PCNA **was** used as an indicator of proliferative activity during liver regeneration. Immunohistochemical detection of PCNA **is** a convenient and commonly used method to grade proliferative activity in various tissues. PCNA expression **is** closely correlated with the S-phase of the cell cycle (17). Fig. 5b **depicts** the PCNA labeling index before partial hepatectomy and at 24 h postoperatively in syngenic and transgenic animals without or with methylspermidine treatment. In resting liver, only about 1% of the hepatocytes **were** PCNA-positive. In syngenic animals, the number of PCNA-positive cells **increased** sharply to over 30% at 24 h after the operation, whereas in transgenic animals, the number of positive cells **remained** at the low preoperative level at this time point (Fig. 5b). Administration of the analogue **did** not change the PCNA labeling index in syngenic livers but dramatically **increased** the number PCNA-positive cells in transgenic livers from about 1% to more than 40% (Fig. 5b).☛We **repeated** the experiment with other groups of syngenic and transgenic rats. In this experiment, PCNA-positive hepatocytes **accounted** for 0.5 ± 0.1% in resting syngenic liver. The number of positive cells **rose** to 10.7 ± 3.5% at 24 h postoperatively without the analogue ($p < 0.001$) and to 9.8 ± 1.5% with the analogue ($p < 0.001$). The corresponding figures for the transgenic animals **were** 0.20 ± 0.0 before the operation, 0.42 ± 0.24% at 24 h postoperatively without methylspermidine, and 25.9 ± 4.9% with methylspermidine ($p < 0.001$).◆It thus **appears** that the analogue completely **reversed** the proliferative block in transgenic livers.

4.7. Discussion (and conclusions)

The main purpose of this section is to relate the new results obtained to the existing knowledge. In many instances, the Discussion section is the most difficult part to write. Discussion is not the place to summarize all the results obtained and to list other's work, especially if they have been described in the Introduction section. If the Introduction contains a formulated question, Discussion is now the place to tell how well the new results answer this question. When published works, including one´s

own, are discussed, the *present tense* is used whereas description of new results obtained the *past tense* is used. The following example shows that both tenses can appear in the same sentence: "*Spermidine serves as a precursor of hypusine [8], but our own present results did not indicate that growth inhibition was mediated through hypusine depletion (Table 1)*." The first part of the sentence refers to a published fact while the latter part refers to one´s own new results presented in the table of the paper.

Subheadings are uncommon in the Discussion section, yet they may occur.

Below is an example of the beginning of an authentic Discussion.

DISCUSSION

The present results strongly **support** the notion that spermidine, and possibly also spermine, **plays** a critical role in the maintenance of pancreatic integrity. In the present transgenic model of pancreatitis, profound spermidine and spermine depletion **was** achieved by the inducible activation of their catabolism. Under the condition of intense activation of polyamine catabolism, depleted pancreatic polyamine pools **could** be replenished by natural polyamines as they would be rapidly degraded without any net tissue accumulation.[2] We therefore **tested** 1-methylspermidine as a substitute for spermidine. Methylspermidine is reported to be metabolically stable as it **is** not a substrate for SSAT and **serves** only as a poor substrate for spermine synthase (18). Moreover, it **appears** to fulfill many of the putative functions of spermidine, such as promoting the conversion of right-handed B-DNA to left-handed Z-DNA (18, 19), serving as the substrate for deoxyhypusine (integral part of eukaryotic initiation factor 5A) and reversing cytostasis caused by inhibitors of polyamine biosynthesis (18, 20). We also **found** that this analogue **was** not an inhibitor of SSAT but **induced** the enzyme in transgenic animals.[3] The fact that methylspermidine **prevented** zinc-induced pancreatitis in transgenic rats **proves** that the profound depletion of the pancreatic polyamines **was** causally related to the development of the organ inflammation and not, for instance, oxidative stress created by polyamine oxidase with its reaction products hydrogen peroxide and aminoaldehyde. The process in which the polyamines **are** required to maintain pancreatic integrity **is** not known. It **is** tempting to speculate that spermidine **acts** through its specific function to serve as precursor for hypusine and the initiation factor 5A (21), especially as intense protein synthesis **is** continuously going on in the pancreas. However, reduction of hypusine content in the absence of sufficient spermidine pool **appears** to be a slow process in which a 50% decrease in hypusine level **takes** nearly a week (22), yet the pancreatitis in the present model **developed** just in 24 h.

It should be noted that the tense of the verbs strictly complies with the sources of the references, i.e. published works or one´s own new results. There are also references to one´s own unpublished results in the text (footnotes 2 and 3), in which the tense of the verbs is the *past* one. Unpublished results never appear in the list of references but are listed in the footnotes like here or appear within parentheses in the text ("*Our unpublished results*"). Some journals (like the example journal) require that unpublished results have their own lists of authors (see the two footnotes at the last page of discussion). In some instances, references to unpublished results can be in the form of personal communications (e.g. "*A. Khomutov, personal communication*"). However, there is a possibility that the journal requires a written approval from the person in question. The Discussion begins with a conclusion, for which support is searched for from published works and from own results. The Discussion continues by offering or excluding mechanistic possibilities, with the aid of which the results are related to the existing knowledge. The last sentence of the above example tends to exclude a certain mechanism by referring to existing literature and to the new results. Note that this sentence contains both *present* and *past tenses* of verbs depending on the source of the reference (published work or own results). Discussion continues:

> As in the case of pancreatitis, methylspermidine **appears** to cover the requirement for spermidine also in rat liver regeneration. The striking induction of the SSAT transgene and profound depletion of hepatic spermidine pool at 24 h after partial hepatectomy **led** to a dramatic block of proliferation, which was, however, equally dramatically **reversed** by the administration of methylspermidine. Many of the experimental findings, such as the early expansion of spermidine pool in regenerating normal liver and the extremely close correlation between spermidine concentration and hepatic proliferative activity (9), **seem** to indicate that spermidine **is** specifically required for the initiation of rat liver regeneration. This view **is** likewise supported by our earlier findings indicating that the maintenance of normal or near normal hepatic spermidine pool in the transgenic animals apparently **occurs** at the cost of spermine, the pool of which **is** reduced by 90% (9). In any event, to our understanding, the present experiments **represent** for the first time a situation in which polyamine depletion has been successfully corrected *in vivo*.
>
> It is highly likely that the use of polyamine analogues or compounds alike **is** not limited to the prevention of pancreatitis in this specific transgenic model as our preliminary experiments have indicated that an activation of polyamine catabolism **is** also involved in other experimental models of pancreatitis. Similarly, the present approach **may** have use in hepatoprotection in case of liver damage.

The above continuation of the discussion now begins to deal with the second part of the title, namely liver regeneration. General conclusions of mechanisms of acute pancreatitis and the possible relationship of the new results to the development of the disease appear at the very end of the Discussion section. The last paragraph of the Discussion contains forward-looking statements about new approaches of drug development. Note that almost all verbs are in the *present tense* in this part of the Discussion. The references to unpublished results with appropriate lists of authors appear in the footnote.

The acknowledgments follow right after the Discussion. According to the style of *Journal of Biological Chemistry* only technical help (and people in general) is thanked here while the grants are listed in the footnote of the title page.

Acknowledgments- We thank Tuula Reponen, Aune Heikkinen, and Sisko Juutinen for skillful technical assistance.

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[2]T.-L. Räsänen, L. Alhonen, R. Sinervirta, T. Keinänen, and J. Jänne, unpublished results.

[3]T.-L. Räsänen, L. Alhonen, R. Sinervirta, T. Keinänen, K.-H. Herzig, S. Suppola, A. R. Khomutov, J. Vepsäläinen, and J. Jänne, unpublished results

Below is an example of a more common Acknowledgments also listing the grant sources.

Acknowledgments
We thank Ms. Tuula Reponen, Aune Heikkinen and Sisko Juutinen for their skillful technical assistance and Dr. Carl W. Porter for the synthesis of DENSPM. This work was supported by grants from the Academy of Finland and from National Institutes of Health Grant CA-76428.

4.8 References

It is imperative that the references strictly comply with the style of the journal, to which the manuscript is aimed. Get familiar with the instructions to the authors, have a recent issue of the journal and check the list of references. As regards the abbreviations of the journals, a general rule is that single-word names ("*Science*") are not abbreviated irrespective of the length of the word ("*Biomedicine*, *Gastroenterology*"). Only published or accepted ("*in press*") papers are listed as references. As indicated

earlier, references to unpublished results and submitted manuscripts appear either in the text (within parentheses) or in footnotes. Depending on the journal, references published as a congress abstracts appear in footnotes, in the text or they may be included as ordinary references. Some journals limit the number of references (e.g. 40). Be careful when preparing the references, as mistakes simply are signs of carelessness. In general, do not cite articles that you have not read. According to a recent analysis, erroneous references frequently are identical indicating that the reference has been copied from somebody's article without reading the original paper. The mentioned analysis came to the conclusion that only 50 % of the cited articles have been read.

Below is an authentic example of erroneous references.

Original article	**Citations**
JÄNNE, J. *J. Biol. Chem*. **246** 1725 (1971)	**464**
JÄNNE, J. *J. Biol. Chem*. **246** 1726 (1971)	**17**
Book chapter	
JÄNNE, J. *Adv. Enzyme Regul*. **24** 125 (1985)	**16**
JÄNNE, J. *Adv. Enzyme Regul*. **24** 125 (1986)	**28**

The examples are an original article, which is readily available, and a book chapter, which is not that easily available. Note that in both cases the faulty references are identical. Erroneous citations (underlined) to the original article comprise only about 4 % of all citations while those to the book chapter greatly outnumber the correct citations. The fact that the faulty references are identical obviously indicate that they are derived from the same erroneous source. In other words, the authors of the erroneous citations have apparently not read the primary paper, but the citation has been picked up from the list of references of someone else.

5. Writing a review article or book chapter

A review article or a book chapter fundamentally differs from a primary paper and does not comply with the IMRAD format. A review article lacks the Materials and Methods as well as the Results sections. The Introduction and Discussion sections

are greatly expanded. At its best, the review article is not an encyclopedic coverage of all possible literature but it critically reviews the existing knowledge and offers new approaches and syntheses of earlier work. A review article is aimed at a much wider readership than a primary publication and hence compromises have to be made with regards to the presentation of detailed information. In practice, the writing of a review article is started by preparing the table of contents and an outline of the article, followed by collecting and reading the primary papers. In most cases, the review article is solicited by a journal but can also be offered to a journal. Many of the journals have "*Reviews Editor*", who is the person to be approached with the writing proposal, possibly by sending a summary of the planned article. The review articles are usually much more cited than the primary papers.

6. Doctoral thesis

In principle, a doctoral thesis is a scientific publication, which, however, can cover (and usually covers) more than one topic and more than one approach to the topic. The monograph-type thesis fully complies with the IMRAD format, yet the Introduction (or Review of the literature) is usually substantially longer than in a primary publication. A thesis based on published papers has features of a primary paper and a review article and in principle complies with the IMRAD format. This thesis format likewise has a relatively long Introduction section while the Materials and Methods as well as the Results sections are shorter due to frequent citations to the original publications. One should be careful with the Abstract as it usually ends up to international distribution ("*Dissertation Abstracts*"). It is an established practice that a thesis contains (usually after the Introduction) a special section called "*The aims of this study*", which in the vast majority of cases has been prepared afterwards. This section, however, is unnecessary as an outline of the aims can be incorporated into the last paragraph of the Introduction (or Review of literature). This section does not belong either in a primary publication or review article but rather in a grant proposal.

In a doctoral thesis based on published articles, there are certain issues, on which the reviewers and public examiner of the thesis will pay special attention. (i) Do the original publications form a logical entity suitable for a doctoral thesis? (ii) The contribution of the author to the original publications (authorships in the original papers).

(iii) The quality of the original publications and the publication forums. (iv) Possible development of entirely new methods and how demanding the methods used are ("state-of-the-art methods"). (v) What is the contribution of the obtained results to the particular field of research and possible scientific breakthroughs. (vi) Acquaintance with the literature. (vii) Maturity and relevance of the discussion. (vii) Linguistic quality. Finally, the public examiner assesses, how well the doctoral candidate defended his/her thesis at the public examination.

7. Poster

A poster is a kind of graphic presentation, in which the author has his/her research project on display. Although extremely common today, the poster tradition is not very old. The poster presentations at national and international scientific meetings became more widely used only in the 1970´s. The poster likewise complies with the IMRAD format, yet graphic considerations and aim to simplicity centrally influence the organization and content of the poster. The Introduction is very short (1 to 2 sentences) clearly stating the aim of the work. Similarly, the experimental section is very short not necessarily describing the individual methods but merely the methodological approaches. Unlike in primary publication, the Results section is the main part of the poster. Short discussion is often replaced by Conclusions in the form of short numbered sentences. The number of references is kept at a minimum. The majority of bad posters are bad because the author tries to present too many matters. If the author of a poster has to explain the content of the poster rather than answering scientific questions, the poster has certainly failed.

8. Verbal presentation

The audience listening to a verbal presentation is more heterogeneous than the readers of a primary publication and the message of a verbal presentation has to be digested in a very short time. Therefore, a verbal presentation should be pitched at more general level than a written publication. The oral presentation starts with the identification of the problem and finishes by offering the solution. The organization and the use of the illustrative material are of central importance for a successful verbal presentation. If the message of a picture does not come across in about five seconds,

the picture is poor. The use of excessive tricks offered by computer programs for slide shows may even cloud the whole message of the picture. The number of slides has to be fit according to the scheduled time, e.g. one slide per every two minutes of the presentation. The fundamental difference between verbal and written presentations is the fact that a written paper contains all methodological details (making it possible to repeat the experiments) but an oral presentation certainly does not. Those who are not native in English can rehearse the pronunciation of American English at Merriam-Webster on-line dictionary (http//www.m-w.com/dictionary.htm).

9. Submission of the manuscript and editorial correspondence

9.1. Publication forum

After the completion of the manuscript, one has to decide which journal the manuscript will be submitted (in fact, this decision has been reached already before starting the writing process in accordance with the Instructions to the Authors). The general aim is to publish the manuscript in a journal as prestigious as possible. On the other hand, a distinct advantage of a less prestigious but more specialized journal is the fact that the latter usually reaches a more expert readership than the former. A clear disadvantage of multidisciplinary journals of high prestige (Science, Nature, PNAS) is the strict space limitations not possibly allowing the presentation of all the results of the study. A prestige factor ("*impact factor*"), which is based on the number of citations directed to the journal articles, has been computed for nearly all journals. The impact factor is derived by dividing the number of citations to the articles of the journal over a period of two years by the total number of articles published during the same time period. The impact factor for journals publishing primary papers may vary between near zero to more than 30. The use of the impact factor as a quality measure for an individual publication has been heavily criticized as, for instance, only one sixth of the total articles of the journal determines its impact factor. The best quality criterion for an individual scientific paper obviously is the number of citations specifically directed to that publication. In any event, the impact factor is going to remain and it will distinctly influence the choosing of the publication forum. Finland is apparently the only country in the world where the impact factor is included in the legislation, as the publication forum has certain influence on the distribution of Government special funds to university hospitals.

Below is the latest list (2004) of impact factors for selected scientific journals publishing mainly primary papers.

Impact factors of selected journals (2004)

New Engl. J. Med.	38.570	PNAS	10.452
Nature	32.182	Blood	9.782
Science	31.853	Diabetes	8.848
Cell	28.389	Mol. Cell. Biol.	7.822
Nat. Genet.	24.695	Cancer Res.	7.690
Nat. Biotechnol.	22.355	Nucleic Acids Res.	7.260
Genes & Develop.	16.385	FASEB J.	6.820
J. Exp. Med.	14.588	J. Biol. Chem.	6.355
Neuron	14.439	Mol. Endocrin.	5.872
J. Clin. Invest.	14.204	Gene Ther.	4.977
Gastroenterology	13.092	Int. J. Cancer	4.416
Circulation	12.563	Biochem. J.	4.278
EMBO J.	10.492	Biochemistry	4.008

A complete list of all journals can be obtained at: http//isi2.isiknowledge. com/ portal.cgi.

9.2. The Editorial Offices of scientific journals

The Editorial Offices of scientific journals mostly consist of elected and paid officials. To the former personnel belong usually the Editor-in-Chief, Editors, associate Editors and the members of Editorial Board. The paid personnel commonly include technical Editors, such as Managing Editor, Desk Editor and Copy Editor. However, with regards to the acceptance of a manuscript for publication, the peer review process and the reviewers occupy a central position. The reviewers may be members of the Editorial Board, members of a larger Editorial Advisory Board or independent scientists (*ad hoc* referees). The latter reviewers are anonymous experts in a particular field of research. The reviewers do not make decisions with regards to the acceptance of the manuscript for publication but their recommendations and suggestions for amendments have an essential impact on the acceptance process. The actual decisions will be made by the Editors of the journal. If the reviewers agree, the decision is easy but, if their opinions are conflicting, the Editor has to decide or ask for a further opinion.

As indicated, the peer review process is intimately involved in the processing of the manuscript for publication. In fact, "peer review" is defined by Merriam-Webster on-line dictionary as "*A process by which something proposed (as for research or publication) is evaluated by a group of experts in the appropriate field*."

Fig. 3 depicts the manuscript in the editorial process.

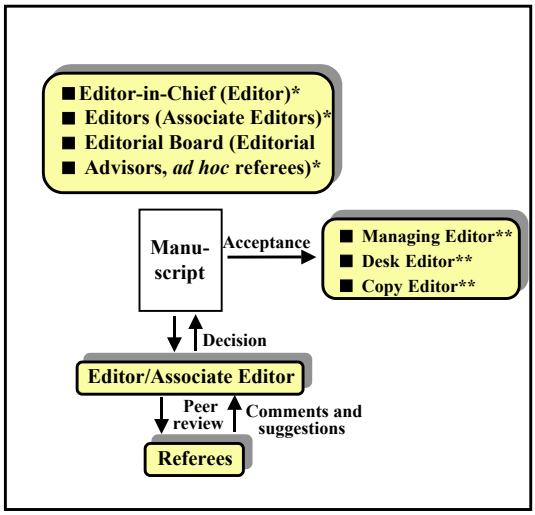


Fig. 3. *The manuscript and the editorial process.*elected, **paid officials*

9.3. Covering letter (cover letter)

When the manuscript is submitted it is always is accompanied by a covering letter. The letter format is usually very simple. Some of the journals require a formal statement that the manuscript has not been published before and is not under consideration for publication: "*This manuscript has not been published before nor it is under consideration for publication elsewhere*". A statement about the authorship may also be required: "*All authors agree upon the content of the manuscript and being listed as authors*". The covering letter also contains a statement regarding the results and their novelty, i.e. why should these results to be published.

Nearly all journals encourage the authors to provide names of suitable referees though these wishes of the authors are not necessarily respected. The authors can likewise suggest names of scientists not to be consulted as reviewers (e.g. existing conflict situation). In principle, such wishes are almost always respected by the journals.

The following is an authentic (the name of the Associate Editor has been omitted) example of a covering letter. As indicated, the author suggests that a given person (the

name is imaginary) is not consulted as a reviewer. Note also that the last sentence of the letter provides a short justification for the publication.

Editorial Office
Journal of Biological Chemistry April 12, 2002

Dear Sirs:

The enclosed manuscript entitled "Targeted disruption of spermidine/spermine N^1-acetyltransferase in mouse embryonic stem cells" by K. Niiranen, M. Pietilä, T.J. Pirttilä, A. Järvinen, M. Halmekytö, V.-P. Korhonen, T.A. Keinänen, L. Alhonen and myself is respectfully submitted for publication in the Journal of Biological Chemistry.

We would appreciate that Dr. James E. Smith is not consulted as a reviewer.

The manuscript provides novel information on the role of SSAT in polyamine homeostasis and sensitivity to polyamine analogues.

Yours sincerely,

Juhani Jänne, M.D., Ph.D.
Professor of Biotechnology

Some journals (e.g. Nature family, Science, FASEB J.) give the opportunity for a "preview" of the manuscript. In that case, the summary of the paper and a covering letter are sent to the Editorial Office for the preview. In many instances, this is not advisable because usually the decision is made by Managing Editor *ex officio* not necessarily consulting the Editors. It is better to send the complete manuscript to the Editor who then decides whether peer review is carried out. Some journals (very few) like to carry out the peer review "double-blindly", in which case the names of the authors and institutions are not disclosed to the referees.

9.4. Criteria for acceptance

In general, the better is the journal, the tougher are the criteria for acceptance. Only about 5 %, depending on the journal, of the manuscripts are accepted "as they stand". Accordingly, critique will be an automatic outcome. In many cases, the journey of the manuscript halts already at the Editorial Office without being sent for review. A manuscript solely describing a phenomenon ("*Effect of something on something*") without any mechanistic implications is very likely to be rejected today.

The primary rejection rate (requiring a new submission) is in good journals well above 50 % while very prestigious journals reject more than 90 % of the manuscripts. Reasons for turning down a manuscript include: "*Insufficient contribution to the field*" or when the manuscript has been sent to a multidisciplinary journal (Science, Nature, PNAS): "*Suits better for a specialized journal*." A more general reason for rejection is the requirement for more experiments. After the rejection of the manuscript, one should seriously consider another journal after taking into consideration the editorial comments received. In case of conditional acceptance, the first thing to do is to clarify whether additional experiments are required or would some formal amendments and condensation of the text suffice. The response to the critique can also contain counter arguments when the authors disagree with the reviewers.

9.5. Editorial response

Below is an authentic editorial response to the manuscript mentioned in the example covering letter.

Dr. Juhani Jänne
A.I. Virtanen Institute for Molecular Sciences
University of Kuopio
P.O. Box 1627
Kuopio, FIN-70211, Finland May 2, 2002

Dear Dr. Jänne:
Your manuscript entitled "Targeted disruption of spermidine/spermine N^1-acetyltransferase in mouse embryonic stem cells" has been reviewed by the Editorial Board. The Reviewing Editor who evaluated the manuscript found that you have created an interesting and novel model system for studying polyamine homeostasis. The results clearly challenge some of the "conventional wisdom" and their publication should be of considerable interest. As you will be note from the accompanying comments, the Reviewing Editor suggests a drastic revision of your Discussion section with a better focus on your novel findings and the way in which they contradict or change previous hypotheses on the subject. When you return to the Journal with a revised manuscript, please provide me a detailed listing of changes made from the original. In addition, in an attempt to stem the growth of the Journal we are asking all authors of potentially acceptable manuscripts to shorten them as much as possible but at least 10%.

Yours sincerely,

For the Editorial Board

As indicated in the editorial letter, the manuscript is conditionally accepted and there is no need for further experiments. The main criticism is directed to the Discussion part of the manuscript that needs to be focused. At the end of the letter, there is a requirement for condensation of the manuscript, which is typically demanded by nearly all journals. The Editor likewise requires a detailed list of changes made in the new version upon resubmitting the revised manuscript. The latter implies that the authors deal in their response with all the points raised by the reviewer (see also the accompanying response to the critique). The editorial correspondence contains the comments of the reviewer to be communicated to the authors (see below).

Comments for authors:

The present studies deal with a mouse embryonic stem cell line in which the SSAT gene was disrupted. Since it is X-linked a single knock-out results in a null phenotype for ES cells with the XY karyotype.
The characterization of the various cell lines is thorough and the interpretation of most of experiments is very clear:

1) The knockout was achieved and the mutant ES cells did not have any SSAT protein or activity. One could suggest that Figures 1 and 2, and Figures 3 and 4 may be combined into single figure (A and B).
2) The data showing the differential growth inhibition of wt and mutant cells by DENSPM are also clear. One may consider omitting Figure 7 since the results can be stated convincingly in the text.
3) The authors suggest that the analogue can replace the natural polyamines (p. 9). Why is it only toxic to wild-type cells?
4) Revise the Discussion section drastically and focus the discussion on the novel experimental findings.
5) The paper could also benefit from another check on the use of English.

In most journals, the reviewers assess the manuscript with regards to the priority of publication and the quality of the manuscript. These assessments are not usually communicated to the authors. On the whole, the evaluation process usually takes weeks, sometimes months. If the process is prolonged very much, it is advisable to consult the Editor with regards to the status of the manuscript. This usually speeds up the review process.

The comments of the anonymous Reviewing Editor (see above) typically begin with a general statement containing a short description of the results and how they have been interpreted. This is followed by detailed comments and suggestions (mainly to condense the manuscript). Finally, a language check is asked for. On the whole, the criticism is clearly benign and is mainly directed to formalities.

If the response to critique and comments has been appropriate, the manuscript is likely to be accepted for publication. However, occasionally the reviewers keep bringing up new comments even after a satisfactory response to the original comments. This represents an inappropriate editorial procedure and may require a direct contact to the responsible Editor. After the final acceptance of the manuscript, the Managing/Desk Editor starts to deal with the technical issues, such as the revision of the language, and may send a number of technical queries, to which the authors must respond carefully. As a final outcome of the technical editing and all the changes, one may end up with the following conclusion: "*Somebody has murdered my prose!*"

9.6. Response to critique

The following example again represents authentic correspondence where the authors present their response to the editorial comments. Note that the overall nuance of the letter is very polite and thanks are expressed for the constructive criticism provided in the editorial letter. The comments of the Reviewing Editor are dealt in the order of their appearance in the editorial correspondence. The changes made in the revised manuscript are described in detail and the places of the changes are indicated. Some journals, but not this one, require that the changes made must be marked (underlining, shadowing, etc.) in the manuscript.

The response to critique satisfied the Editor, and the manuscript was immediately accepted for publication.

The editorial process ends with proofs, which the corresponding author most likely receives as a PDF attachment sent via E-mail. This is the final opportunity to make changes (small) in the text. The queries of the Managing Editor are usually shown in the proofs and should be properly dealt with. The proofs are accompanied by reprint order form that should be filled in. The form usually also contains page charge, if any, and charge for color illustrations.

Associate Editor
Journal of Biological Chemistry May 7, 2002

Re.: Manuscript M2:03599 (Niiranen et al.)

Dear Sir:

Thank you for your editorial letter of May 2, 2002 and the accompanying extremely thorough and sound criticism provided by the Reviewing Editor. We now enclose a revised manuscript, in which we have addressed the concerns of the Reviewer to our best understanding.
The comments of the Reviewer (in order of their appearance) have been dealt as follows:

1. The original Figures 1 and 2 have been combined (new Figure 1A and B) as well as Figures 3 and 4 (new Figure 2A and B).
2. The original Fig. 7 has been omitted and the data presented in the text.
3. The description of Fig. 3 (old Fig. 5) (page 9) is modified to indicate that the analogue "could replace the natural polyamines from their intracellular binding sites" that would be the major reason for polyamine depletion, not the induction of SSAT. The discussion concerning the mechanisms of analogue cytotoxicity has also been modified (page 12) to offer a third possibility, in which analogues exert their cytotoxicity independently both of SSAT and polyamine depletion.
4. The discussion has been focused and condensed as required.
5. The linguistic mistakes have been corrected.

Due to changes made, we believe that the manuscript is now condensed by more than 10%. While hoping that the manuscript now is acceptable for publication, we thank again for the sound and very constructive criticism provided in your editorial letter.

9.7. Electronic submission

Nearly all major journals offer the possibility (and most of them encourage or even require) to submit the manuscript electronically. Electronic submission may, in some cases, dramatically shorten the editorial process. At best, the manuscript may be accepted for publication in a few days. This is, however, extremely unlikely as the most time consuming part of the process is the peer review that takes its own time.

The journals and journal families have their own systems for electronic submission, yet these are very similar. All the communication takes place through E-mail. Most of the journals prefer to receive the main body of the text and the illustrative material as a single PDF file and the covering letter as a separate file (pasted in the submission form). The PDF file can also be created as a part of the submission process. Today, almost all journals accept graphics and pictures only as TIFF or EPS files and hence the graphics programs and the statistical software must have an option to save the material as these files. Apparently, the PowerPoint files will become acceptable in the near future (some publishing houses accept them already).

9.8 Publication or patent?

Publishing of the results prevents the patenting in Europe. Publishing is not limited to a scientific article but likewise covers any public release, such as oral presentations, congress abstracts, posters, etc. In the U.S.A. (and Canada), a patent application can be filed within 12 months after the public release and in Japan within 6 months. Patenting is very expensive and it advisable to leave the compiling of the patent application for professional people.

10. Linguistic pitfalls and useful phrases

This chapter contains a collection of English words and phrases, which may create problems at least to those who are not native in the English language. The chapter likewise contains useful phrases picked up from scientific literature that can be used to enliven the text and to avoid repetition of phrases.

10.1. Misused words

"*Which/that*", the difference between the words is that *which* describes but *that* determines. Examples: "*Mice, which are transgenic, also have shorter tails*" and "*Mice that are transgenic also have shorter tails*." Note the difference between the sentences. The first sentence means that all mice are transgenic whereas the latter sentence means that only some mice are transgenic. Similarly, the use of the words "*while/whereas*" may create difficulties. *While* implies a strict temporal linkage (at the same time as). Incorrect usage: "*I started the experiment while she finished it*." Cor-

rect usage: "*Caesar fiddled while Rome was in fire*." "*Needless to say*" why then to say. "*Remarkable*" is not a synonym for "*marked*", it is more. "*Employ*" (used as a synonym for "*use*") implies salary payment. "*Parameter*" use "*variable*." Use "*relatively*" only when comparing. "*Filled symbols*" not "*solid symbols*". "*Quite*" is quite unnecessary. "*Varying*" is often used in the meaning of "*various*". "*Before*" is better than "*prior to*" (the latter is commonly used by Americans). "*In the present communication*" use "*here*". "*As can be seen from Fig. 2, growth is more rapid*", make it simpler and use an active voice: "*Growth is more rapid (Fig. 2)*" or "*Fig. 2 shows that growth is more rapid*". "*Significant*", use only when related to statistical analyses. "*From the standpoint of*" read "*according to*." "*Approximately*" better "*about*." "*In comparison with*" not "*in comparison to*." "*Begin or start*" are better than "*commence*" (the latter is commonly used by Americans). "*Considerable amount of*" actually means "*much*". "*Created the possibility*" replace by "*Made possible*", "*Enabled (a person)*" or "*Allowed (an action)*," "*Murine*" means rat or mouse, not only mouse. "*Usage*" is not synonym for "*use*". The former has a very limited use: "*Language usage*" whereas "*use*" is "*widely used*,"

10.2. Singular/plural and numbers

"*About 10 g <u>was</u> added*" not "*About 10 g <u>were</u> added*" as only one unit was added. If the addition would occur in doses of 1 g then "*About 10 g <u>were</u> added*." Latin-derived words ending with "a" ("*data, media*") are plural: "*Data are expressed as means ± S.D.*" Numbers 1 to 9 are written as words, 10 and up as numbers. "*Three experiments*" and "*13 experiments*." Exception, when a number is followed by a unit: "*3 ml*" and "*13 ml*." A sentence is not started with a number: "*Reagent A (5 ml) was added*." When a number is written as a word, the following unit is not abbreviated: "*Five milliliters of reagent A was added*."

10.3. Nouns as adjectives/adjectives as nouns

The following is a bad example of the use of several consecutive nouns as adjectives. "*Rat liver polyamine oxidase activity*." "*Hepatic disease*" is better than "*Liver disease*" and "*Administration of drug*" is better than "*Drug administration*." Latin-derived words "*in vitro, in vivo, de novo*" are not used as adjectives. "*Tests in vitro*" not "*In vitro tests*." The word "*supernatant*" is usually an adjective: "*100,000 x g su-*

pernatant fraction" is better than "*100,000 x g supernatant*." Natural genotype is "*wild type*" but when used as an adjective "*wild-type mice*."

10.4. Abbreviations

Abbreviations are never used in the title and their use should also be avoided in the Summary. The word or phrase is first written in full following abbreviation in parenthesis: "*Ornithine decarboxylase (ODC)."* Many journals allow the use of abbreviations only when the word or phrase appears in the text five or more times. The journals usually have an "official" list of abbreviations (RNA, DNA, ATP etc), which can be used without writing the word first in full. Abbreviations can be avoided by using pronouns ("*it, they, them*") or substitutive expressions ("*the inhibitor, the substrate, the drug*"). The abbreviation "*i.e.*" means *that is* whereas "*e.g.*" means *for example*.

Units are abbreviated when used with numbers ("*4 mg was added*") and the abbreviation is the same in the singular and plural. Units are not abbreviated when used without numbers: "*Specific activity is expressed as nanomoles of GTP incorporated per milligram of protein per minute*."

Note the use of indefinite article: "*A Master of Science degree*" but "*an M.Sci. degree*." The latter abbreviation is read as "*em ess*" (begins with a vowel). The generic names of organisms are first written in full "*Staphylococcus aureus*" and subsequently abbreviated "*S. aureus*."

University degrees are written in American English with dots: "*M.Sc., M.D., Ph.D.*" whereas in British English without dots: "*MSc, MD, PhD*."

10.5. British versus American English

The spelling of many words is slightly different in British and American English. In most instances, the American spelling is usually a bit simpler. British "*ou*" is replaced by "*o*" in American English: "*tumour*" vs. "*tumor*", "*behaviour*" vs. "*behavior*." Similarly, the British "*ae*" is replaced by "*e*" in American English: "*anaemia*" vs. "*anemia*," "anaesthesia" vs. "anesthesia." The British double l "*ll*" is usually replaced by single l "*l*". "*labelled*" vs. "*labeled*", "*signalling*" vs "*signaling*." The last two words are typically misspelled when one tries to write American English. A typical difference is also the use of the endings "*re*" and "*er*", of which the former represents British English: "*centre*" vs. "*center*", "*litre*" vs. "*liter*." In British English, cer-

tain verbs contain "*ys*" corresponding the American "*yz*": "*analyse, catalyse, dialyse*" vs. "*analyze, catalyze, dialyze*." Finally, an interesting difference: "*In British English periods and commas are outside the quotations marks"*. "*In American English they are inside the quotation marks.*" When writing English, be consistent with the variation.

10.6. Greek alphabets

As the Greek alphabets are frequently used in scientific writing, their tabulation helps a lot for finding the correct symbol. The table below lists Greek alphabets, their pronunciation and the corresponding Roman alphabet.

Greek alphabets:

Α α	alpha	a	Ν ν	nu	n
Β β	beta	b	Ξ ξ	xi	x
Γ γ	gamma	g,n	Ο ο	omicron	o
Δ δ	delta	d	Π π	pi	p
Ε ε	epsilon	e	Ρ ρ	rho	r,rh
Ζ ζ	zeta	z	Σ σ	sigma	s
Η η	eta	ê	Τ τ	tau	t
Θ θ	theta	th	Υ υ	upsilon	y,u
Ι ι	iota	i	Φ φ	phi	ph
Κ κ	kappa	k	Χ χ	chi	ch
Λ λ	lambda	l	Ψ ψ	psi	ps
Μ μ	mu	m	Ω ω	omega	ô

Familiar examples are*: α-adrenergic, β-sheet, DNA polymerase β, β-casein, λ-phage, ΔM, Ω-factor, χ-square*.

10.7. Useful phrases

The scientific literature is packed with useful phrases, which can be used to enliven one's text and a to avoid repetitions. The following examples have been picked up from the scientific literature by the author over a period of two decades. The genes of capsule: "*The genes governing capsule synthesis*…" It is known: "*It is now common knowledge that* ..." Current interest: "…*are currently focused on*…" Matched; "*Is compatible with life*." In spite of a lot of work: "*Despite the expenditure of a great deal of effort*…" We know little: "*Our knowledge of this crucial subject is still in its*

infancy…". Experimental limitations: "*The limitations inherent in the experimental method employed.*." Caution should be exercised: "*There is reason for caution in interpretation*…" More distrust: "*There is growing suspicion that*…" Avoid difficulties: "*This difficulty can be circumvented by*…" Ungrounded conclusions: "*Unwarranted mechanistic implications*…" Is known: "*Awareness of the events*…" Not certain: "*There is no assurance that replacing* Mg^{++} *by polyamine can*…" Uncertain results: "*The result should be considered suggestive, at best, since the group studied was small*." Something has to be done before: "*A more global view of the subject should be obtained before embarking on such an undertaking*," Not fully proven: "*Lack of categorical proof*." Not proven: "*…is unproven on present evidence*." Weak conclusions: "*The validity of these conclusions is in serious doubt due to two main factors*…" Restrictions: "*But within such critical limitations, these studies did indicate that*…" Causing: "*A direct cause-effect sequence*…" Warranted: "*It is based on reasonable logic*." Doubtful: "*There is good reason to suspect that*…" Drug doses: "*…occurs in clinically relevant dosage ranges*…" Similarly: "*In analogous fashion*." Doubtful mechanism: "*The mechanism involved remains the subject of controversy*." Not obvious: "*It is not immediately apparent how this would lead*…" Unclear: "*…is still a matter of debate*." Bad planning: "*Suffering greatly from poor design*," Most important issues: "*…only to summarize salient features*…" Limit the discussion: "*…and confine discussion to their possible functions*." Return to the beginning: "*Let me refer back to the beginning of this review*." Uncertain: "*We are by no means certain that*…" Remove of differences: "*Differences were minimized or abolished*." One can ask: "*The question can be posed only as to*…" Doubtful results: "*…remains clouded by reports of conflicting data*." Hopefully tells: "*It is hoped that this overview depicts the status of our current knowledge*." Answers only after we know: "*The answers to these and other questions may come only after we have much better perspective about how membrane transport occurs at molecular level*." Challenging lack of knowledge: "*This lack of knowledge should not be looked upon negatively but seen instead as a challenge*." Incomplete data: "*…although data are not as complete as one might wish*." Unsuccessful: "*…would appear to be doomed to failure*." Getting worse: "*…appear to make a bad situation immeasurably worse*." Not dealt with specifically: "*A matter not specifically addressed in this review is the question as to what extent*…" Two enzymes act together: "*Through the concerted action of two enzymes*." Not expected: "*Lack of significant progress would be the expected outcome*." Sup-

ports the view: "*Has lent credence to the view*." Conclusions: "*Three other conclusions derived from this experiment*." Weak evidence: "*The stringency of the evidence varies in quality*." Not an extensive coverage: "*The reader should not expect and encyclopedic coverage of the entire literature, but a treatise of selected topics*." Preliminary evidence: "*The accumulated evidence is still tentative*." Soon: "*…followed not long thereafter*". Focused on transgenic mice: "*In this review particular attention will be devoted to recent transgenic mouse experiments that…*" The last example approaches already the borders of good sense of style: "*Once the wrinkles in the technique have been ironed out, researchers…*"

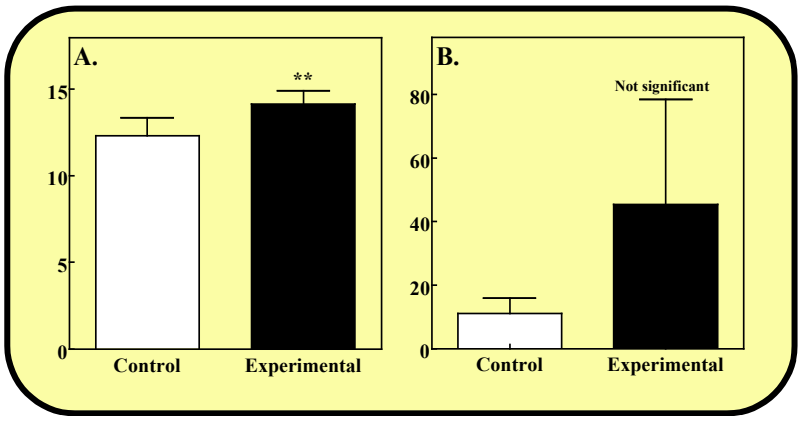
11. Tips for statistical analyses

Statistical analyses occupy a central position in the preparation of scientific article and the significances of differences (or better the lack of those) may profoundly influence the acceptance of the paper. It is not the intention of this text to deal with basics of the statistics, but rather give practical tips for statistical analyses. The performance of statistical analyses today is very easy, as a large number of statistical software packages are available. These packages usually are so user-friendly that a researcher with no formal statistical training easily can perform the analyses. However, there are certain basic issues that have to be understood when carrying out the analyses in general and interpreting the results in particular. For the example analyses, I have chosen GraphPad Prism (GraphPad Software, Inc., San Diego, CA, U.S.A.) software package. This package is extremely user-friendly with very good graphics. Only those statistical tests that I have needed during my research career are included.

11.1. Before statistical analyses

A central issue in the interpretation of analyses and experimental results is the fact that statistical significance is not equal to biological or clinical importance. A statistically highly significant difference between experimental groups does not automatically mean that this difference would be biologically or clinically relevant. For instance, a decrease in a few millimeters of mercury of systolic blood pressure in response to treatment may be statistically highly significant, yet it hardly has any clinical relevance. Unfortunately, the statistical significance of a difference is often em-

phasized without considering its practical relevance. On the other hand, even a very large difference between experimental groups but without statistical significance may create problems in publishing the results. The enclosed example would hopefully illustrate the problem. In the figure A, the difference between the two groups is minimal, yet highly significant. In figure B, the difference between the groups is many-



fold but not statistically significant. I would believe the case B and increase the sample size, e.g. number of animals per group.

11.2 Variability: Standard deviation or standard error of the mean?

Standard deviation (S.D.) quantifies the scatter, i.e. the variability between values. In principal, the change of S.D. cannot be predicted upon increase in the sample size (yet, it usually decreases). Standard error of the mean (S.E.M.) indicates how accurately the true mean of the population is known. S.E.M. decreases when the sample size increases. If the scatter is attributable to biological variation, S.D. is used. In systems *in vitro* with no biological variability (series of enzyme assays, for instance), S.E.M. is used as the scatter is caused by experimental imprecision. Some scientists exclusively use S.E.M. as it is always smaller than S.D. (S.E.M. = S.D./ n). The common belief that one should be consistent and use either S.D. or S.E.M. is not necessarily true as the source of the variability, biological or methodological imprecision, determines their use.

11.3. Outlier (abnormal value)

An experimental value that is far from the other values is called an outlier. There may be trivial reasons for abnormal values, such as incorrect data entry into a computer or funny-looking test tube or strange animal etc. In such cases the abnormal value can be corrected or omitted. However, the possibility remains that the outlier is

derived from entirely different population (e.g. sick animal among healthy population). Statistically it is possible to detect whether the value is derived from different population or distribution and omit it "legally". The value is standardized by dividing the difference between the mean value and the outlier by scatter (S.D.) yielding Z value. This is called Grubbs´ test (see the enclosed example). In Gaussian distribution, only 5 % of the values are more than 1.96 x S.D. from the mean. If the Z value is more than 1.96, the abnormal value is an outlier ($p < 0.05$) and comes from different population. If N is small (see the table) the critical value is less than 1.96. After computing Z value, compare the value with critical values of the table at given N. If the

Z table

N	Z
3	1.15
4	1.48
5	1.71
6	1.89
7	2.02
8	2.13
9	2.21
10	2.29
11	2.34
12	2.41
13	2.46
14	2.51
15	2.55
16	2.59
17	2.62
18	2.65
19	2.68
20	2.71
30	2.91
50	3.13
100	3.38

Example

Fat percentage of the mice: 2.12, 1.80, 2.09, 2.28, 2.47 and 4.1
Mean ± S.D.:
2.48 ± 0.83 (N=6)
Is 4.1 an "outlier"?

$$Z = \frac{[2.48-4.1]}{0.83} = 1.95$$

Critical value for Z (N=6) is 1.89, accordingly 4.1 is an "outlier"

value is greater than the critical value, the abnormal value is derived from a different population with the probability of $p < 0.05$.

11.4. Comparing two groups: Parametric tests: *t* test

The name of the *t* test is derived from a British statistician who wrote under the pseudonym "Student" ("*Student's test"*). The test is by far the most common statistical test used to compare two means. In fact, the test analyses whether a difference between two means differs from zero. The significance is influenced by the magnitude of the difference and the scatter within the groups.

Paired *t* test: Paired *t* test is used when a variable is measured before and after some intervention (e.g. drug) in the same person or animal. The test is likewise used when patients are enrolled pair-wise: same age, equal severity of the disease etc. One of the

pairs will be treated, the other not. Similarly, the paired test will be used when a laboratory experiment is repeated several times with control and experimental group.

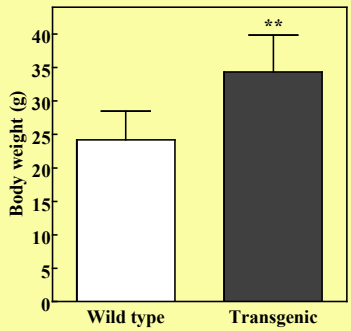
Unpaired *t* test: This test is used when two independent groups are compared. For instance, two animal groups, of which one group is exposed to a drug and the other group remains untreated.

t Test can be either one-tailed or two-tailed. The former uses only one of the tails of the Gaussian distribution whereas both tails are used in the latter. One-tailed test can only be used when there is justifiable (prior) knowledge that the mean of one group is larger or smaller than that of the other. In comparison with the two-tailed test, this test gives p values that are half of those of the two-tailed test. The two-tailed test is used in the vast majority of analyses. Below is an example of the performance of unpaired *t* test.

Parameter	Value	
Table Analyzed	t-test	
Column A	Wild type	
	vs	
Column B	Transgenic	
Unpaired t test		
P value	0.0051	
P value summary		**
Are means signif. different? (P < 0.05)		Yes
One- or two-tailed P value?		Two-tailed
t, df	t=3.564 df=10	
How big is the difference?		
Mean ± SEM of column A		24.17 ± 1.759 N=6
Mean ± SEM of column B		34.33 ± 2.246 N=6
Difference between means		-10.17 ± 2.853
95% confidence interval		-16.52 to -3.810
R squared	0.5595	
F test to compare variances		
F,DFn, Dfd	1.630, 5, 5	
P value	0.6048	
P value summary		ns
Are variances significantly different?		No

Example:

- **Mouse weight (g):**
- **Wild type: 23, 31, 21, 27, 24 ja 19**
- **Transgenic: 36, 29, 27, 34, 41 ja 39**



t Test is a parametric test, which assumes that both groups follow bell-shaped Gaussian distribution (normal distribution) i.e. the variances (S.D.^{2}) of the groups do not differ significantly. The computer program in the enclosed example, as most of the statistical programs, automatically compares the variances (F test) and reports any significant difference between them. As shown, the variances in the example do not differ significantly. If the variances are significantly different (or the data are ranked), nonparametric tests must be used. Giving certain significant limits (*p < 0.05, **p < 0.01 and ***p < 0.001) for p values dates back the to era without comput-

ers. The exact value of p (p = 0.0051 in the example) can be given as well. Parametric tests are more powerful (smaller p values) than nonparametric tests. Before the use of nonparametric tests, the data can be transformed (reciprocal, logarithmic) to possibly achieve normal distribution.

11.5. Nonparametric tests: Mann-Whitney test

As mentioned, parametric tests cannot be used if the variances differ significantly or the data are ranked. Below is an example of the former i.e. variances differ significantly.

Example:

- Compare two groups (A and B)
- Perform *t*-test

Group A, value	33	24	9	32	6
Group B, value	1	8	7	4	5

t-test

Unpaired test	
P value	0.0261
P value summary	*
Are means signif. different	Yes
One-or two-tailed P value	Two-tailed
t, df	t=2.724 df=8
F test to compare variances	
F, DFn, Dfd	21.43, 4.4
P value	0.0058
P value summary	**
Are variances significantly different	Yes

As seen, F test finds a significant difference between the variances of the two means excluding the use of *t* test. As the groups in the example are independent, the nonparametric test of choice is Mann-Whitney test. The test ranks all the values from

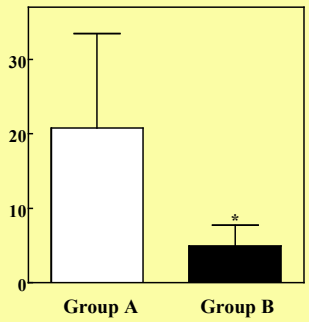
Example:

- The test ranks the values

Group A, value	33	24	9	32	6	
Group A, rank	10	8	7	9	4	38
Group B, value	1	8	7	4	5	
Group B, rank	1	6	5	2	3	17

Mann-Whitney test

P value	0.0317
P value summary	*
Are medians significantly different (p<0.05)	Yes
One-or two-tailed P value	Two-tailed
Sum of ranks in column A,B	38,17



low to high (paying no attention to the different groups). The smallest value gets the rank of one and the largest value the rank of N (total number of the values in the two groups). The test computes the sum of ranks for both groups. The larger the difference is between the groups, the smaller p becomes. The test is not suitable for very small groups as if the total sample size is less than seven the p value becomes automatically larger than 0.05 irrespective of the difference between the means. Even though non-parametric tests are less powerful than parametric tests, nonparametric tests have the advantage that they are insensitive to outliers because absolute values are not used. Accordingly, outliers may remain. Although in the example, the values were ranked manually, this is not necessary as the program does it automatically. Absolute values are entered into the computer. Note that the p value is substantially higher than in the *t* test.

11.6. Nonparametric tests: Wilcoxon test

This test corresponds paired *t* test and is also called *matched pairs test*. This test computes the absolute differences between each pair, ranks them from low to high and sums the ranks. If the difference between the rank sums is large, the p value is small. Finally, the test computes whether the medians of the groups differ significantly from each other.

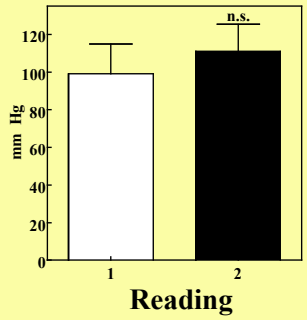
Example:

Systolic blood pressure (mm Hg)

Person	Reading 1	Reading 2	Difference	
A	120	110	-10	2
B	80	95	15	3
C	90	120	30	5
D	110	130	20	4
E	95	100	5	1

Wilcoxon test

P value	0.1875
P value summary	ns
Are medians significantly different (p<0.05)?	No
One- or two tailed P value?	Two-tailed
Sum of negative, positive ranks	-2.000, 13.00



11.7. Comparing three or more groups: One-way analysis of variance (ANOVA)

As mentioned, *t* test and the corresponding nonparametric tests can only be used to compare two different groups. In case that there are three or more groups, it is illegitimate to use *t* test to compare control group, for instance, separately with each experimental group. The proper test for three and more independent groups is analysis of variance (ANOVA). If there is only one grouping variable, the test is called one-way ANOVA. ANOVA is a parametric test that assumes a Gaussian distribution. With two groups one-way ANOVA is equal to *t* test. With three or more independent groups, ANOVA only reports whether there is statistically significant difference between the groups but does not compare individual groups. For multiple or pair-wise comparisons, several *post hoc* test are available.

Below is an example of the performance of one-way ANOVA.

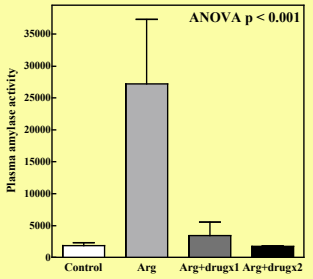
Example:

■ **Effect of arginine on plasma amylase activity**

Control	Arg	Arg+drugx1	Arg+drugx2
2309	15580	2059	1698
1410	32040	2020	1798
1800	33980	6529	1715
		3168	

One-way ANOVA

P value	0.0003
P value summary	***
Are means significantly different?	Yes
Number of groups	4
F	19.51
R squared	0.8667



As indicated in the example, ANOVA finds out a significant difference between the four groups but does not compare individual groups.

11.8. Post hoc tests for ANOVA

Post hoc tests can only be used if ANOVA detects an overall significant difference between the groups. All the *post hoc* tests are variations of *t* test.

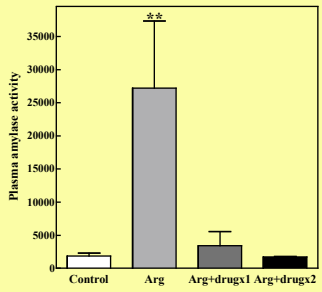
Dunnett's test: Compares control (or any individual) group with other groups.

Bonferroni test: Compares selected pairs of groups or all pairs of groups. The distinct disadvantage of Bonferroni test is the fact that the test is very conservative (large p values). This does not matter when the number of the groups is small, but large num-

ber (more than five) of groups creates problems. If the limit of significance has been set to $p < 0.05$ this will be divided by the number of comparisons (e.g. in case of 10

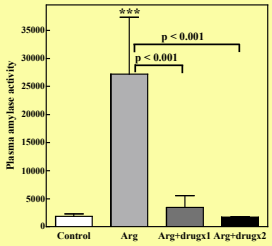
Dunnett´s *post hoc* test

	Mean diff.	P value
Control vs Arg	-25360	p<0.01
Control vs Arg+drugx1	-1604	p>0.05
Control vs Arg+drugx2	102.7	p>0.05



Bonferroni´s *post hoc* test

	Mean diff.	t	P value
Control vs Arg	-25360	6.306	p < 0.001
Control vs Arg+drugx1	1604	0.4264	p > 0.05
Control vs Arg+drugx2	102.7	0.02553	p > 0.05
Arg vs Arg+drugx1	23760	6.315	p < 0.001
Arg vs Arg+drugx2	25460	6.331	p < 0.001
Arg+drugx1 vs Arg+drugx2	1707	0.4537	p > 0.05



comparisons, the limit of significance decreases to $p < 0.005$). In the following exam ple of Dunnett's post *hoc test* the control group has been compared with experimental groups and it significantly differs only from the *Arg* group. In the example of the Bonferroni test, all groups have been pair-wise compared with each other.

The Bonferroni test indicates that both of the drug-treated groups differ highly significantly from the untreated (*Arg*) group.

Tukey and Newman-Keuls tests are nearly identical further *post hoc* tests for ANOVA. They are related to the Bonferroni test.

11.9. Nonparametric tests: Kruskal-Wallis test

This test is used for comparison of three or more independent groups when the data are either ranked or the variances of the groups differ significantly from each other. As in other nonparametric test, each value is ranked so that the smallest value gets the rank of one and largest value the rank of N (total number of observations). The ranks are summed. The larger the difference of rank sums is, the smaller p value. The test is also called "*Kruskal-Wallis one-way ANOVA by ranks*".

11.10. Nonparametric tests: Friedman test

This test is used for comparisons of three or more paired groups. The principle of the test is identical to that of Kruskal-Wallis, i.e. the pairs are ranked and the ranks are summed for each group. The test is also called "*Friedman two-way ANOVA by ranks (repeated measures)*".

11.11. Two-way ANOVA

The test determines how a given response is influenced by two different variables, i.e. there are two grouping variables (in one-way ANOVA only one grouping variable). For example, the effect of three different drugs on persons of different gender. The test answers three questions: (i) Are the average responses identical to all drugs? (ii) Are the responses same in men and women? (iii) Do the factors interact: is the difference between men and women same with all drugs?

So-called "Repeated-measures two-way ANOVA" is a convenient test when the effect of a drug is followed as the function of time, for instance. The measurements are repeated continuously as the function of time in the absence or presence of the drug. The test finds out whether the curves differ significantly from each other. The curves can also be compared by computing the areas under the curves ("*AUC*"). Repeated-measures two-way ANOVA can also be used when persons are enrolled as matched pairs (age, gender, disease etc) or when a laboratory experiment is repeated several times (with control and treated groups). The difference between ordinary ANOVA and repeated-measures ANOVA is the same as the difference between unpaired and paired *t* test, i.e. the latter is more powerful (smaller p values).

The following example depicts an experiment where cell growth has been followed for four days in the absence or presence of a potentially cytotoxic drug. The

Two-way ANOVA
(repeated measures)

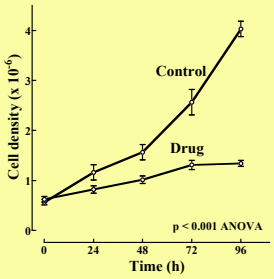
Source of variation	% of total variation	P value
Interaction	22.99	p < 0.001
Time	53.47	p < 0.001
Column factor (drug)	22.47	p < 0.001

Example:

Does the column factor affect the result? (*Are the curves different?)* The P value is < 0.001. The effect is considered extremely significant.

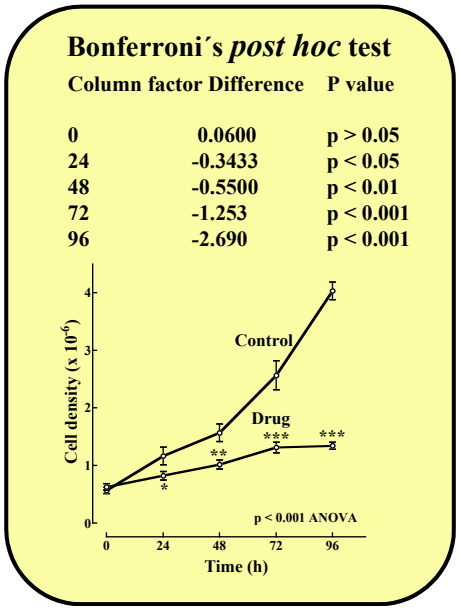
■ **Cell densities**

Time (h)	Control	+Drug
0	0.62;0.58;0.51	0.69;0.58;0.62
24	1.2;1.0;1.3	0.9;0.82;0.75
48	1.4;1.6;1.7	0.95;1.0;1.1
72	2.8;2.6;2.3	1.32;1.22;1.40
96	4.0;3.9;4.2	1.28;1.40;1.35



samples have been taken as triplicates at each time point. The analysis reveals that the curves differ highly significantly, i.e. the drug significantly inhibits cell growth. The

significance of the differences at each time point could be computed by using unpaired *t* test but it can also be tested using the Bonferroni post hoc test as shown in the example below. There is a significant difference between all time points except the first ones.



11.12. Linear regression

Linear regression analyzes the relationship between two variables (X and Y). The test finds out the best straight line through the data points. The course of the line is determined by the slope and the intercept (at Y or X-axis). The line can also be used as a standard curve to obtain new values of X from Y or Y from X. It is possible to transform nonlinear data to obtain a linear relationship. Familiar examples are Lineweaver-Burk plot (reaction velocity versus the reciprocal of substrate concentration; v versus 1/[S]) and Scatchard transformation for ligand binding affinity (bound/free ligand versus bound ligand).

The analysis computes r^2 value, which indicates the "goodness" of the fit. The value is without dimensions between 0 (horizontal line) and 1.0 (all points on the line). In the best fit, the line minimizes the sum of squares of the vertical distances of the points from the line. F test computes the p value for the relationship by taking into account the r^2 value and the number of points.

The strange name (regression) is apparently derived from the first applications of the analysis where lengths of fathers and their sons were compared. The results of the

analyses revealed that the sons of tall fathers were shorter than their fathers. In other words, the sons of the tall fathers were “regressed”.

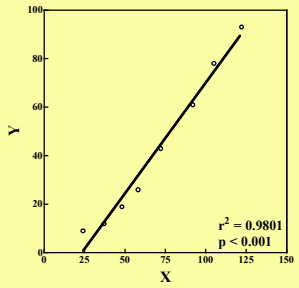
As indicated in the example analysis below, a very close ($r^2 = 0.9801$) and highly significant ($p < 0.001$) correlation exists between X and Y.

Example:

X	Y
24	9
37	12
48	19
58	26
72	43
92	61
105	78
122	93

Best-fit values

Slope 0.9139 ± 0.05313
Y-intercept when X=0.0 -21.12 ± 4.080
X-intercept when Y=0.0 23.11
1/slope 1.094
95% Confidence Intervals
Slope 0.7839 to 1.044
Y-intercept when X=0.0 -31.10 to -11.14
X-intercept when Y=0.0 13.95 to 30.34
Goodness of Fit
r squared 0.9801
Sy.x 4.826
Is slope significantly non-zero?
F 295.9
DFn, DFd 1.000, 6.000
P value < 0.0001
Deviation from zero? Significant



11.13 Contingency tables

Contingency tables compare categorical variables, e.g. dead versus alive, disease versus no disease, artery obstructed versus artery open. The results of a given experiment may not be fully accordant with the theoretical distribution and the question is posed as to whether the difference between the expected and observed distribution is greater than that caused by random variation. The test used are χ^2 (Chi squared) test and its modifications. The simplest contingency table is a 2 x 2 table (two variables determining the two columns and two rows). The columns determine the outcomes and the rows the groups. In the following example, in which the effect on mortality of a drug has been tested, the variables are wild-type versus transgenic animals and the categorical variables are dead or alive The data in this kind of table can be analyzed with χ^2 test, for which Yate´s continuity correction can be adapted or using Fisher’s

exact test. The latter gives the exact value for p and is hence recommendable unless the sample size is very large (thousands).

Below is an example of a 2 x 2 contingency table. Note that the p values for each modification are slightly different.

Example:

■ **Effect of drug**

Group	Dead	Alive
Wild type	18	12
Transgenic	8	22

Fischer´s exact test

P value	0.0182
P value summary	*
One- or two-sided	Two-sided

Chi-square

Chi-square, df	6.787, 1
P value	0.0092
P value summary	**
One- or two-sided	Two-sided

Chi-square with Yate´s correction

Chi-square, df	5.498, 1
P value	0.0190
P value summary	*
One- or two-sided	Two-sided

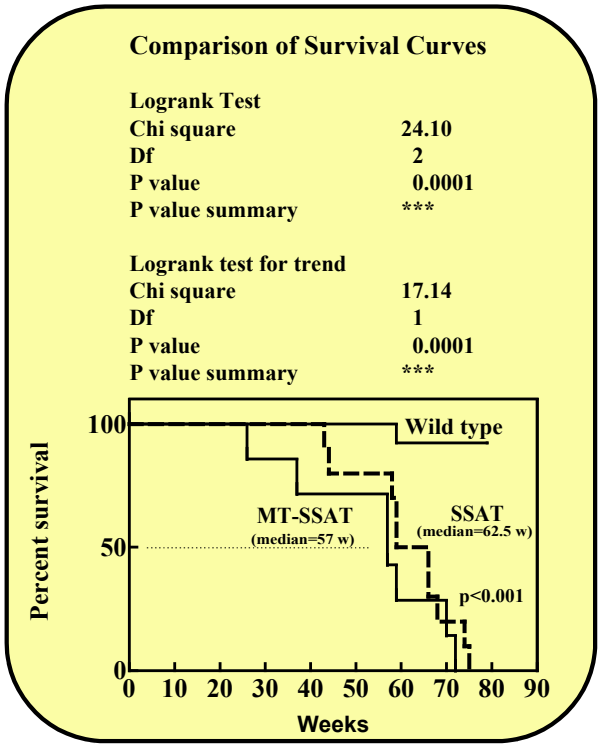
11.14 Survival curves (Kaplan-Meier)

In many experiments, the end point is death or any event occurring only once (rejection of a transplant, obstruction of an artery etc). The end point does not need to be negative (discharge from hospital, graduation etc). Kaplan-Meier survival curves determine the occurrence of the end point as the function of time. In GraphPad Prism program, data is entered in such a way that X represents the time (week, month) until the end point. The end point is entered as Y = 1 at the corresponding time. Any drop-out (out of control) is entered as Y = 0 (censored data). For instance, if a survival experiment is concluded after a certain time period, all animals still alive will appear as Y = 0 at the last time point of observation. The following example shows the entry of the data derived from a survival experiment. The total observation period was 79 weeks. Of 12 control mice (WT), one died at week 59, the remaining mice were still alive at the time the experiment was concluded (week 79). All mice of the two transgenic groups (MT-SSAT and SSAT) died before the end of the observation period. Logrank test is used to compare two or more groups. The test compares at each time point the expected and observed number of end points, collects the values into a χ^2

(Chi square) table and computes the p value. In case of three or more groups, the test calculates the trend.

The examples below show the entry of the data and the results of a survival experiment consisting of three groups.

X Week	Y1 WT	Y2 MT-SSAT	Y3 SSAT
59	1		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
79	0		
26		1	
37		1	
57		1	
57		1	
59		1	
70		1	
72		1	
43			1
44			1
58			1
59			1
59			1
66			1
66			1
68			1
74			1
75			1



11.15. Choosing the statistical analysis

Fig. 4 outlines the selection of the statistical tests for two (A) or more (B) groups. The first question would be the type of analysis, i.e. whether parametric or non-parametric analyses will be used. If the variances of the groups are significantly different or the data are ranked, parametric tests are excluded and nonparametric tests should be used. Categorical variables require the use of contingency tables or Kaplan-Meier survival curves, if there is a time function. Searching for correlation between the variables implies the use of linear regression. Unpaired or paired *t* tests are used for the analysis of two groups with Gaussian (normal) distribution whereas groups with ranked data or unequal variances will be analyzed with Mann-Whitney test (unpaired data) or Wilcoxon test (paired data). For three or more groups with Gaussian distribution, ANOVA will be the analysis of choice. One-way ANOVA is used when there is only one grouping variable and two-way ANOVA when there are two group-

ing variables. The corresponding nonparametric tests for three or more groups are Kruskal-Wallis test (unpaired data) and Friedman test (paired data).

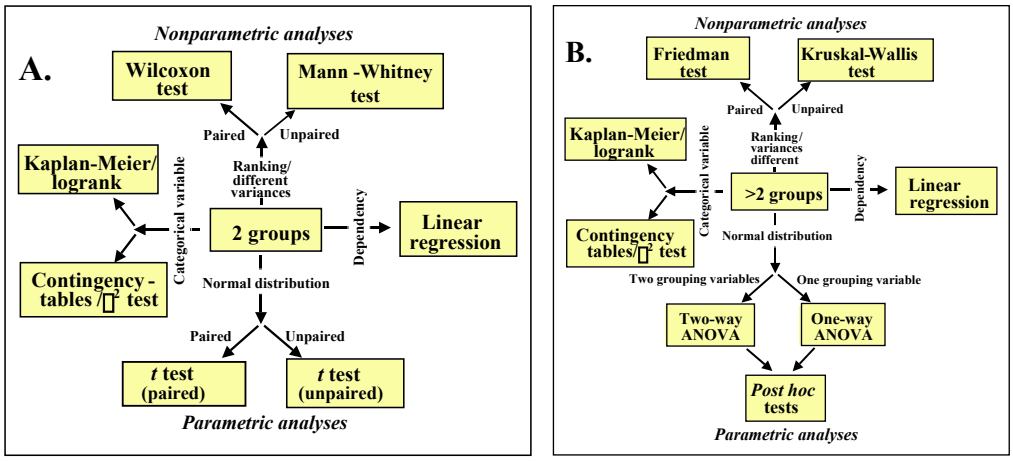


Fig. 4. *Choosing the statistical test. A, two groups; B, three or more groups*.

12. Writing a grant proposal

Writing a successful grant proposal is probably one of the most creative but also most demanding tasks for a scientist. A grant proposal combines in its research plan earlier and present work with new ideas and hypotheses. A grant proposal contains almost all central features of science, such as creative conceptualization, detailed experimental and budgetary planning, projection and analysis of the anticipated results and even a rescue plan for unexpected outcome. Even though a scientific paper and a grant proposal have much in common, there is a fundamental difference between them: the former tends to sell *obtained results* whereas the latter tends to sell new *research ideas*. This fact profoundly influences the preparation of a grant proposal as credibility, i.e. that the applicant is able to complete the proposed research, becomes a central issue.

12.1. Organization of the research plan

Research plan is by far the most important part of the grant proposal. Nearly all funding agencies use a very similar format for the research plan, which largely has been adapted from that used by the National Institutes of Health (NIH) in the U.S.A. The research plan usually consists of the following components:

1. *Abstract (Summary)*

2. *Background and significance*
3. *Objectives, approaches and methods*
4. *Research group and resources*
5. *Results*
6. *Budget and budget justification*

The length of the research plan is most commonly limited to 10 pages. Most funding agencies are very strict in this respect.

12.2. Title

The title should describe the central aim or concept of the proposed project. It should neither be too specific nor too general. In the latter case, feasibility of the proposal can be doubted (especially if the funding period is short). The reviewers for the proposal are usually selected on the basis of the title and/or the Summary. The title should be short giving a good description of the proposal.

12.3. Abstract (Summary)

The Abstract is probably the most important part of the research plan as the reviewers of the proposal read this first and if there are large number of applications, the Abstract may be the only part of the proposal to be read. The Abstract is likewise used to select the appropriate reviewers for the proposal. The Abstract of a research plan is not equal to the Summary of a primary paper. The project abstract describes *what will be done and why it is important* and not *what has been done and why it was important*. The Abstract does not contain results, though the previous work of the applicant in the field should be strongly emphasized. The Abstract almost never is longer than one printed page.

A proper organization of the Abstract is very important. Relate the proposal to a broader context and show possible gaps in current knowledge: "*Pancreatitis is a life-threatening disease, the exact pathophysiology of which is largely unknown*." Describe your earlier work relevant to the proposal: "*We recently developed a transgenic rat model for acute pancreatitis that closely resembles the human necrotizing disease*." Present the research problems and hypotheses to be tested. "*The main aim of the present proposal is test whether treatment modalities effective in our transgenic model are also applicable to other models of acute pancreatitis*." Describe where and how the study will be carried out and what kind of methodological approaches will be

used: " *The study will be carried out at the A.I. Virtanen Institute for Molecular Sciences, University of Kuopio. The analogues proven to be effective in our transgenic model will be tested in cerulein and arginine-induced pancreatitis. The effect of the drugs will be assessed by general mortality rate, histopathology and plasma amylase activity*." Finally, assess the potential significance of the results in a broader context and do not be too modest: "*This study will be of unique value in designing new treatment modalities for acute pancreatitis*."

12.4. Background and significance

This section resembles the Introduction of a primary paper but it is structured in a slightly different way. Place the proposal in a broader context by citing the most important publications in the field in general and your own previous published work relevant to the proposal in particular. It is of utmost importance to bring up your preliminary (unpublished) experiments supporting the proposal and giving credibility to it. You may present these preliminary experiments under a special subheading: "*Own preliminary results*." It is, however, highly advisable to strictly adhere to the truth as this part commonly contains scientific exaggeration and conclusions that are based on qualitatively or quantitatively poor preliminary experiments. Finally, you assess the general contribution of the results to be obtained to this particular field of research ("*Significance*").

12.5. Objectives, approaches and methods

The most important aim of this section is to convince the reviewers and the funding agency that the applicant has a realistic and feasible research plan. The sequence *objectives-approaches-methods* stepwise focuses the performance of the study from central aims to specific methods. *Objectives* represent the general aims of the study in the particular field of research. *Approaches* represent the strategic use of techniques and *methods* stand for the use of specific methods. The credibility of the research plan is strengthened by giving an appropriate reference for each method and even more by stating that the method is already in routine use in the laboratory. The proposed methods should naturally be relevant to the resources of the laboratory (see also *Research group and resources*).

This section also contains the timetable with possible milestones. The inclusion of a time schedule of the study belongs to the credibility issues and also helps the appli-

cant to outline the performance of the study and its progression. As an example of an entirely unrealistic research plan was a proposal to generate gene-disrupted mice within one-year funding period in a laboratory with no prior experience. Setting up milestones within the funding period likewise strengthen the credibility of the research plan. The milestones can easily be presented in a form of time chart.

This section also contain ethical issues, such as the appropriate approval of animal experiments (*Institutional Animal Care and Use Committee*) and approval of human trials (*Institutional Review Board and Informed Consent*). It should be noted that the latter approval is not only limited to clinical trials but should also be obtained for analyses of samples derived from patients.

Some funding agencies (Academy of Finland, for instance) require that this section contains goals for post-graduate training and hence a list of post-graduate students aiming to the doctoral degree should be provided.

12.6. Research group and resources

This section might as well appear under the *Objectives, approaches and methods*, in fact it mostly does, but some funding agencies (e.g. Academy of Finland) like it as separate section. Here, the human resources, i.e. the composition of the research group and special expertise of each member of the group are described. The distribution of work among the members of the group is likewise sketched. This is the place to present external expertise needed for the study. It should be noted, however, that most funding agencies require a written agreement from the collaborators. Listing the materialistic resources (equipment, laboratory space, cell cultures, animal facilities, special reagents etc) is important as it, together with used methods, helps to assess the feasibility of the proposal.

12.7. Results

In this section, the anticipated outcome of the results is assessed. The expected results may be highly concrete, such as a creation of transgenic animals or development of a specific therapy etc or they may be of more general nature, such as generation of new knowledge in the field. The general importance of the expected new results to the field is also assessed here and how to further exploit them. One should be also prepared for unexpected or even unlikely results and have a "rescue plan" ready for any changes in the objectives. The planned publication forums ("*The results will*

be published at the best publication forums") and patenting issues likewise belong to this section.

12.8. Budget and budget justification

A detailed budget is mostly presented in a specific form provided by the funding agency. The research plan usually contains only a budget summary and especially its justification. If the proposed research is very labor intensive, then a large salary budget is justified. On the other hand, if the proposal involves large number of animal experiments, cell culture experiments or expensive special reagents (such as needed in molecular biology), then a large supply budget is justified. In many cases, it is possible to apply funds for pieces of small equipment. If much travel (scientific congresses, meetings with external collaborators etc) is involved, it is advisable to include travel expenses in the original budget as changing the budget items afterwards may create problems or at least a permission from the funding agency is required. Finally, it is highly likely that the sum applied for is not received in full.

12.9. References

The references affect the overall length of the research plan with its page limit. Therefore the number of references should be kept to a minimum. The style of the reference list and how to insert the references in the text is usually not specifically defined by the agency. It is advisable to choose a style that occupies as little space as possible, i.e.. insert the references as numbers in the text in order of their appearance. To save space, the titles of the references can be omitted from list, though occasionally they may be of use to the reviewer. As in case with the primary paper, one should be careful with the correctness of the references.

12.10. Curriculum vitae

Curriculum vitae (CV) and list of publications (complete or covering a certain period of time) are practically always required as appendices to the grant proposal. With regards to the list of publications, some funding agencies require a definite grouping of the publications in the list. Peer reviewed primary publications usually come first followed by book chapters, textbooks etc. It is not advisable to include submitted (not yet accepted) manuscripts in the list unless they are highly relevant to the research plan.

The personnel register maintained by Universities may be formally official but it is not very reader-friendly and is probably written in the wrong language. In practice, all funding agencies accept an informal CV in English language, which does not need to be officially certified (notary public). Below is an example of a CV mostly used by Americans.

CURRICULUM VITAE

NAME:

BORN:
Date and place

PROFESSIONAL ADDRESS:

EDUCATION:

PROFESSIONAL EXPERIENCE:
Chronological or reverse chronological order

RESEARCH INTEREST:

EDITORIAL DUTIES:
Member of Editorial Board:
Editorial Advisor:
***Ad hoc* Referee:**

MEMBERSHIPS AND AWARDS:

MAJOR GRANT SUPPORT:

PARTICIPATION AS INVITED SPEAKER AT INTERNATIONAL SCIENTIFIC MEETINGS:

TEACHING EXPERIENCE:
Courses given:
Doctoral theses supervised:

Name and date of birth are self-explanatory. Some times marital status is also included. **PROFESSIONAL ADDRESS**: Department, University and all contact information (phone, fax, E-mail, cell/mobile phone). **EDUCATION**: Graduated from School, Master of Science (M.Sci.); Licentiate of Medicine (M.D., qualifying examination) or just M.D.; Doctor of Philosophy (Ph.D.); Doctor of Medical Sciences corresponds usually M.D., Ph.D.; Docent or possibly Senior Lecturer. **PROFESSIONAL EXPERIENCE**: Positions held in chronological or reverse chronological order. Instructor; Research Associate; Postdoctoral fellow; Lecturer or Reader (British); Associate Professor; Professor; Dean; Member of the Board; Chairman; Chief Executive Officer (CEO). **RESEARCH INTEREST**: Three to five research topics: Transgenic animals; Molecular Endocrinology; Cancer research. **EDITORIAL DUTIES**: Editor; Associate Editor; Member of Editorial Board; Editorial Advisor; *Ad hoc* referee (list of journals served). **MEMBERSHIPS AND AWARDS**: List of Scientific Societies; Scientific and civil awards. **MAJOR GRANT SUPPORT**: List of grants and sources (years) with or

without actual sums. **PARTICIPATION AS INVITED SPEAKER AT INTERNATIONAL SCIENTIFIC MEETINGS**: List of invited presentations: place, title and year. Also poster presentations can be listed (younger colleagues). **TEACHING EXPERIENCE**: Courses given and theses (M.Sci./Ph.D.) supervised (name, title of thesis, university and year).

12.11. Evaluation of a grant application

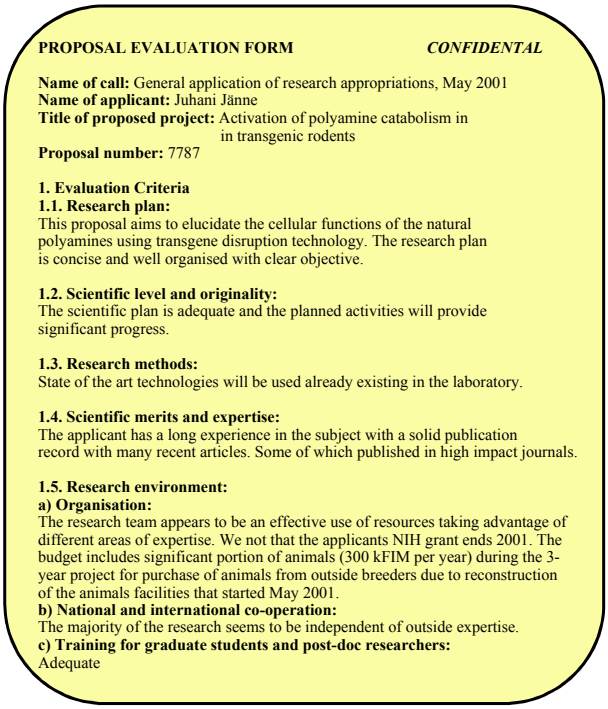
Typical features of a poor grant proposal are carelessness, unrealistic proposal, unfocussed proposal and "scientific whitewash". Carelessness in preparing the proposal is usually shown as disregard for the instructions provided by the funding agency. Unrealistic and unfocussed are almost synonyms meaning that there is no way to complete the proposed research within the funding period or there are far too many research topics needing, for instance, 200 people and 20 years to complete the study. "Scientific whitewash" simply means that the description of preliminary (unpublished) experiments is greatly exaggerated and the conclusions are entirely unwarranted. It is, however, highly advisable to adhere to the truth as otherwise problems may be encountered in the context of the final reporting.

In principle, international panels of experts should be used to evaluate the proposals (the applications have to be written in English). The applicant should receive a written evaluation report. The research plan (and its feasibility) has to be considered as the most important part of the proposal and not the track record of the applicant.

The following is an authentic evaluation report of a grant proposal. As indicated in the evaluation form, the research plan occupies a central position, as it should, in the evaluation. The fact that most methods are already in the use in the laboratory is appreciated. Moreover, the report assesses the research environment and, exceptionally, comments the financial situation of the applicant (an international grant is just about to expire). The high cost for animals and reasons for that are likewise understood. Note that the application did not call for outside expertise. The training for graduate students and postdoctoral fellows is also commented.

The second part of the evaluation report contains the overall evaluation and the ranking of the application. The scores are from 1 (poor) to 5 (outstanding). In practice, funding agencies like the Academy of Finland mostly finance only outstanding and a few excellent applications. Note that a short explanation is provided for each score.

Below are the both parts of the evaluation report. The proposal apparently got funded by the Academy of Finland.



PROPOSAL EVALUATION FORM *CONFIDENTAL*

Name of call: General application of research appropriations, May 2001
Name of applicant: Juhani Jänne
Title of proposed project: Activation of polyamine catabolism in in transgenic rodents
Proposal number: 7787

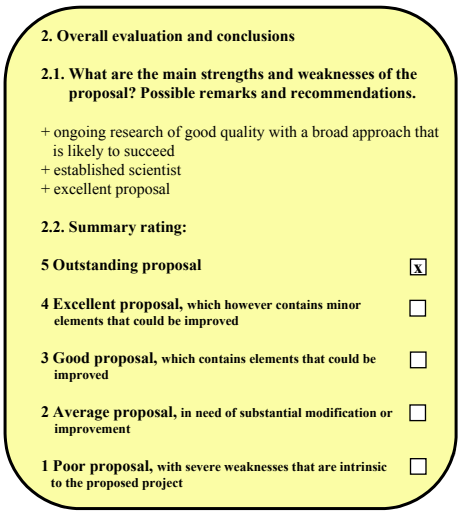
1. Evaluation Criteria
1.1. Research plan:
This proposal aims to elucidate the cellular functions of the natural polyamines using transgene disruption technology. The research plan is concise and well organised with clear objective.

1.2. Scientific level and originality:
The scientific plan is adequate and the planned activities will provide significant progress.

1.3. Research methods:
State of the art technologies will be used already existing in the laboratory.

1.4. Scientific merits and expertise:
The applicant has a long experience in the subject with a solid publication record with many recent articles. Some of which published in high impact journals.

1.5. Research environment:
a) Organisation:
The research team appears to be an effective use of resources taking advantage of different areas of expertise. We not that the applicants NIH grant ends 2001. The budget includes significant portion of animals (300 kFIM per year) during the 3-year project for purchase of animals from outside breeders due to reconstruction of the animals facilities that started May 2001.
b) National and international co-operation:
The majority of the research seems to be independent of outside expertise.
c) Training for graduate students and post-doc researchers:
Adequate



2. Overall evaluation and conclusions

2.1. What are the main strengths and weaknesses of the proposal? Possible remarks and recommendations.

+ ongoing research of good quality with a broad approach that is likely to succeed
+ established scientist
+ excellent proposal

2.2. Summary rating:

5 Outstanding proposal [x]

4 Excellent proposal, which however contains minor elements that could be improved [ ]

3 Good proposal, which contains elements that could be improved [ ]

2 Average proposal, in need of substantial modification or improvement [ ]

1 Poor proposal, with severe weaknesses that are intrinsic to the proposed project [ ]

12.12. Personal grant

The grant proposal is principally prepared according to the described format. Detailed budget, however, is unnecessary providing that funding agency does not require accounting. The agency mostly sends a notification of the grant to the local Internal Revenue Service Office (Tax Office). Currently, a person can receive personal grants worth about € 15,000 per year without paying taxes.

12.13. Scientific writing and the society

Fig. 1 (page 7) depicted the place of an individual researcher in the internal process of science. On the other hand, a scientist has a distinct role also in the interactions between science and society. Funding decisions made by the major agencies are usually public and it is possible that the media and general public become interested in a recipient of a substantial grant. In this case, an individual scientist may possibly generate marked public arousal. In the context of important scientific publications, a scientist may give a press release describing the significance of his/her results. Public awareness is subsequently reached through the media. More seldom, an interesting publication is picked up from its original source leading to an interview of the scientist and finally to the awareness of the general public. The extent of the publicity en-

countering scientists, however, is modest in comparison with that of athletic celebrities. This exemplified by the following story. A researcher was wondering about the extensive public attention and worship paid to the top athletes. His colleague burst out: “Who on earth would pay to see a scientist.”

Fig. 5 summarizes the role of an individual scientist in the interaction between science and society.

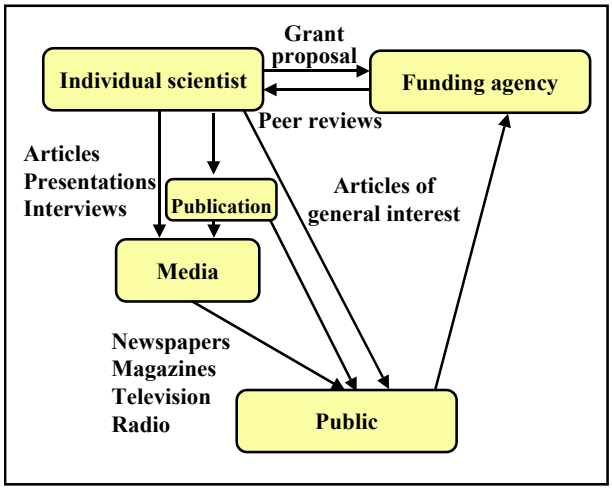


Fig. 5. *An individual scientist and the society*

13. The ethics of research

Research has its own ethical rules, the violation of which may even lead to criminal actions. Scientific work differs from many other civil activities in the sense that a researcher has a remarkable freedom to do his/her work. In many cases, it is exceedingly difficult to verify the original experiments. The latter may lead to the temptation to exaggerate or even to falsify results. However, science effectively controls its own activities, as practically all observations will be repeated and verified. Experimental results that cannot be verified by others simply “die” and disappear from the scientific literature. Hence, there is no need to kill bad science as it will die by itself. In the twilight zone of good scientific practice, there are a number of bad procedures that are, if not condemnable, at least reproachable. “Salami science”, i.e. the experimental data are sliced to pieces that are called “the least publishable units”, distinctly belongs to the latter procedures. Unfortunately, this sort of practice is more rule than exception in the Nordic Countries, as doctoral theses have to contain a certain minimum of original publications leading to a fragmentation of research entities. A further exam-

ple is dual publication that, however, violates good scientific practice at the worst. Dual publication means that same experimental results will be published more than once. It is acceptable that the data have been presented at scientific meetings and published as a meeting abstract in the proceedings but even this needs to be mentioned in the primary paper. In some rare cases, the same data can be published twice providing that the approaches are different and the original paper is properly cited. The use of same control material in several publications without a reference to the original paper is similarly forbidden. Entirely condemnable is to submit the same manuscript (or experimental data) simultaneously to two different journals. The possibility to get caught is substantial, as in highly specialized fields there are only a few competent reviewers and the two manuscripts may end up with the same reviewer.

13.1. Violations of good scientific practice

The National Committee for Research Ethics has in 1998 defined the violations of good scientific practice essentially as listed below:

1. Not giving appropriate credit to someone else´s work
2. Inappropriate citing to published literature
3. Misleading reporting of experimental results/methods
4. Defective entry of experimental results
5. Dual publication of experimental data
6. Public misrepresentation of one´s own result

The two first violations are in all likelihood the most common, though it may difficult to define whether the practice has been condemnable or just reproachable.

The following is an authentic example of a quotation of literature without appropriate reference. Similarities between both texts have been bolded. It is noteworthy that the latter text contains identical references exactly in the same places as in the original text. The original text (2002) appeared in the Introduction section and latter text (2004) in the Discussion section. If not clear plagiarism, it is very close to it.
Probably the most common form of plagiarism is “autoplagiarism”, meaning that the author carefreely cites his/her earlier text in a copy-paste manner.

Niiranen *et al*. (2002) *J. Biol. Chem*. **277**, 25323-25328.

The oxidative catabolism of the higher polyamines spermidine and spermine is accomplished by the concerted action of two different enzymes, namely spermidine/spermine N^1-acetyltransferase (SSAT)[1] and polyamine oxidase (PAO). Cytosolic SSAT N^1-acetylates both spermidine and spermine whereafter they serve as substrates for peroxisomal PAO (1). As PAO strongly prefers acetylated polyamines to the unmodified polyamines as its substrates, SSAT is generally considered as the rate-controlling enzyme in the back-conversion of spermidine and spermine (2). The final product of the …….
References
(1) Hölttä, 1977 (2) Casero and Pegg 1993

Chen *et al*. (2004) *J. Cell Biol*. **167**, 161-170.

The oxidative catabolism of the higher order **polyamines, spermidine and spermine, is accomplished by the concerted action of two different enzymes, SSAT and polyamine oxidase (PAO). Cytosolic SSAT N^1-acetylates both spermidine and spermine,** which then **serve as substrates for peroxisomal PAO (Holtta, 1977).** Because **PAO strongly prefers acetylated polyamines to unmodified polyamines, SSAT is generally considered the rate-controlling enzyme in the back conversion of** the higher order polyamines, spermidine and spermine, to the lower order polyamine, putrescine **(Casero and Pegg, 1993).**

13.2. Scientific fraud

The following malpractices distinctly fulfill the criteria for scientific fraud:

1. Data fabrication
2. Misrepresentation and falsification of data in such a way that the results based on observations will change
3. Misappropriation of somebody's original idea, research plan or observations
4. Plagiarizing somebody's research plan, manuscript, article or other text or part of it

As indicated, violations of good scientific practice apply to scientific publication and grant proposal as well.

13.3. Procedures in suspected scientific fraud

National Committee for Research Ethics has given clear instructions on how to proceed in case of suspected fraud (summarized in Fig. 6). An example could be a situation where a collaborator has not been included as an author although he/she had centrally contributed to the results of the publication. When scientific fraud is suspected, a formal letter describing the case is sent to the Rector of the University of the suspect (or to the Director of a Research Institute). The Rector or the Director decides whether a preliminary hearing is needed. If the Rector or Director decides that a preliminary hearing is not necessary, the case will be cancelled. However, if the plaintiff is not satisfied with the decision, he/she can bring the case to the National Committee for Research Ethics and ask for an opinion.

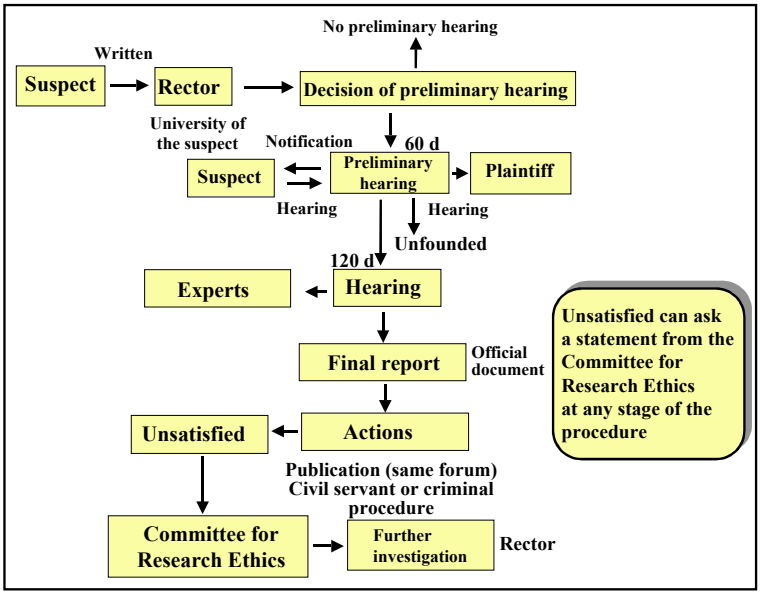


Fig.6. *Procedures in suspected scientific fraud*

A decision of preliminary hearing has to be reached in 60 days. During the preliminary hearing, the suspect is interrogated and the plaintiff will be informed about the hearing. The decision of a possible hearing has to be reached in 120 days. The hearing is carried out by experts in the particular field of research. After the hearing, the experts produce a final report, which is an official document. Depending on the results of the hearing, the case is either cancelled or further actions are taken. The action may be a publication in the same forum (retraction) or civil servant or even criminal procedure. It is noteworthy for those unsatisfied with the results, that the National Committee for Research Ethics can be contacted at any stage of the procedure.

The Committee can return the case to the appropriate Rector or Director for further investigation.

The number of cases of scientific fraud in Finland has been very low, but as the competition is all the time tightening up, it is expected that at least milder cases are showing up and hence one should be prepared to react properly.

14. Further readings

Robert A. Day, ***How to write and publish a scientific paper***, 5th edition, Oryx Press 1998, Phoenix, Arizona.

Andrew J. Friedland and Carol L. Folt, ***Writing successful science proposal***, Yale University Press 2000, New Haven & London.

Harvey Motulsky, ***Intuitive biostatistics***, Oxford University Press 1995, New York & London.