4a

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Hypotheses

Having established the problem and a logical structure within which it can be considered, one or more specific hypotheses should be stated. This is the most important part of the Specific Aims section, and is often missing or stated in such general terms as to be useless. Unless a specific hypothesis can be stated and tested, research is nothing more than a fishing expedition. Admittedly, descriptive research begins the study of a new field and is essential as a base for in-depth studies, but there are very few such new fields of study. A trap that awaits us all is the "interesting observation" that beckons to us like the Sirens of Ulysses. Research that is designed to investigate something just because it is there may be very interesting to the PI, but rarely to enough of the Study Section to generate a fundable priority score. Phenomenological proposals are weak and tend to end up on the rocks. It is worth repeating that a proposal is strengthened if a hypothesis is clearly identified, if it relates logically to a broad theoretic model, and if the proposed experiments will actually test it.

Some hypotheses are hardly worthy of the name. "Colorectal cancers are detected more often with the flexible sigmoidoscope than with the rigid sigmoidoscope" is a hypothesis of sorts, but is trivial and hardly worth a research effort to test. It was proposed as the basis for a retrospective study of data from over 800 sigmoidoscopies in a large county

hospital. The flexible instrument reaches higher into the colon than the rigid sigmoidoscope. The difference in detectability could be related to variation of the incidence of cancer with position along the colon, a possible problem of epidemiology. This proposal was greatly strengthened when it was rewritten as an epidemiological study to test the hypothesis that the incidence of high colonic cancer in men is reduced by long-term use of a high-fiber diet. The same data were used for the study, but the approach was changed.

We live in an age of powerful experimental tools. The availability of a technology has the tendency to stimulate research that uses it. Proposals that are based on technological advances rather than on important hypotheses cannot help but be weak. A good example was the introduction of a powerful procedure used by molecular biologists called the polymerase chain reaction, or PCR. This procedure amplifies minute amounts of DNA in a tissue section, thereby permitting the recognition of virus particles. With the emphasis on AIDS research, there was a rush to seek evidence of HIV in a variety of different tissues. "The hypothesis to be tested is that HIV is present in the [you name it] of patients with AIDS Related Complex" was a formula for far too many studies, some of which were actually funded since the NIH was compelled to use the funds given to it by Congress for AIDS research. A hypothesis is not strong unless it is related to a significant theoretical model of the disease.

A hypothesis worthy of consideration can be tested directly or gives rise to corollaries or predictions that can be tested. Untestable hypotheses are worse than useless; they are destructive in that they may consume time and effort without a concomitant advance of knowledge.

Excessive listing of hypotheses signals lack of focus. A single important hypothesis is best; most proposals list two or perhaps three (four is one too many).

Specific Aims (Tests of the Hypotheses)

Specific Aims are then stated. These are the tests of the hypotheses presented in terms of experiments or groups of experiments. These should be listed numerically and should be reiterated verbatim and in order in the Experimental Design and Methods section of the proposal. The Specific Aims should be just that, specific. They must be brief and indicate the general nature of the technology used (but should not include discussion of the actual methods). This section usually fills about a third of the

page. The reason for each of the aims should be obvious from consideration of the hypotheses and their corollaries. There is never enough room on this page to really explain the rationale of each aim. But this is done in exhaustive detail later in the proposal. It is only necessary that each aim fit within the structure of the theory. Avoid editorializing: "These studies may lead to the development of novel strategies for the treatment of whatever." Do not cite references. If the reviewer has to look up a reference in order to get through the Specific Aims, it is a failure. Three Specific Aims are usually enough!

Example

The following Specific Aims was submitted to the NIH. It is instructive for several reasons. You might wish to evaluate and rate it before reading the critique of it.

> Alzheimer's disease (AD) is a dementing disorder of unknown etiology. The diagnosis of "presumed" or "probable" AD is made through clinical diagnosis, in recognition that AD can only be definitively diagnosed histopathologically. Characteristically, memory is initially impaired, followed by visuo-spatial deficits, and, finally, involvement of all cognitive functions (Hutton, 1987).
>
> We hope to address a number of Specific Aims by the completion of this project:
>
> 1. Is there selective involvement of a particular component or class of cells in the visual system of AD patients? If so, can this be related to the pathophysiology of AD in the rest of the brain? If there is a predilection for loss of a class of ganglion cells in AD, this may yield insight to the reasons for predominant degeneration of large neurons in other areas of the brain (Terry et al, 1981).
> 2. Can visual testing be used, in conjunction with present neurological and psychometric evaluations, as a screening procedure to identify AD?
> 3. Can visual testing or histopathological assessments of the visual system be used to identify subtypes of AD? If so, this might provide insights leading to possible management and treatment strategies for AD.
> 4. We will gain insights into both anatomical and functional AD subgroups through correlative histopathological and clinical assessments of the visual system in the age-matched controls (normals) used in this study.
> 5. Significant new data relevant to the effect of age on the visual system will be gathered.

Critique

This is a weak Specific Aims. The first line is excellent, but the rest of the opening paragraph is fluff without a clear relationship to the proposal.

The brief description of the defects of AD speaks down to the reviewers, who are certainly well informed about it. The reference is superfluous.

There is no hypothesis or theory offered.

To write, "We hope to do this or that," is weak. It may be honest, but it is bad grantsmanship. It leaves room for doubt as to whether what follows will be achieved. A major concern of the reviewer is the question of what the PI will have left if part of the proposal does not work. The Specific Aims should never contain anything that is controversial, equivocal, or negative.

Aim 1 could have been stated as a hypothesis and test combined. "We will test the hypothesis that large neurons are selectively destroyed in AD by measuring the sizes of ganglion cells in the retinas of AD patients and of age-matched controls." The rest of Aim 1 is editorializing. The suggestion that this retinal study might be correlated with the results of other research on brain tissue is speculation. Such correlations are notoriously difficult, and to throw one in here seems to be window dressing. Speculations in the Specific Aims are very destructive since they interfere with its purpose, which is to provide an executive summary of the project. Speculation is by its very nature weak and argumentative. The less of it in a proposal the better.

Specific Aim 2 is clearly a non sequitur. What does a screening procedure have to do with large cell loss? Actually there is an association, but it is speculative. There is a suggestion that visuomotor skills are dependent on large ganglion cell input from the retina to the brain. The investigators hope to find visuomotor deficits in AD patients, and if these can be seen early in the disease, the tests could be used for screening. Unfortunately, the opening paragraph states that memory loss is the initial event in AD, and loss of visuomotor function comes later. Clearly a test of memory loss would be a better screening procedure. This is not suggested since there is no apparent correlation between memory loss and large cell loss.

Specific Aim 2 could have been stated thus: "A corollary of this hypothesis suggests that large ganglion cell dependent visuomotor function of AD patients should be defective. We will test this with eye track recordings in patients and age-matched controls."

Specific Aim 3 is combined speculation and window dressing. What is meant by "subtypes of AD"? At present, as stated in the opening paragraph, we cannot even diagnose AD without histopathology, so how can we talk about clinical subtypes? Of course diagnosis by histopathology

cannot help "management and treatment strategies of AD." This aim is best eliminated.

Specific Aim 4 assumes that Aim 3 was successful, and is otherwise editorializing, as is Aim 5. Both should be eliminated.

A restructuring of this Specific Aims could be built around the following, excerpted from above and expanded:

> Alzheimer's disease (AD) is a dementing disorder of unknown etiology. Recent studies have shown that AD is associated with loss of larger brain cells and with optic nerve degeneration. Since the retina is actually part of the brain and has been studied in far greater functional and anatomic detail, it may provide an ideal model in which to investigate the relationship of a cell's size to its susceptibility to damage in AD.
>
> Specific Aims:
>
> 1. We will test the hypothesis that large neurons are selectively destroyed in AD by measuring the sizes of ganglion cells in the retinas of AD patients and in age-matched controls.
> 2. A corollary of this hypothesis suggests that large ganglion cell dependent visuomotor function of AD patients should be defective. We will test this with eye track recordings in patients and in age-matched controls.

This is considerably less than a full page. It should be expanded to emphasize the desirability of a collaborative study involving a basic scientist and a clinician, and the availability of a large patient base. Ideally, the theoretical model would contain hypotheses about the functional relations between AD etiology and neuron size, or about the mechanisms that drive these relations. These should lead to the prediction that there should be a predilection of AD to affect large rather than small neurons.

A successful Specific Aims section can be read in about 3 minutes. It leads the reader to understand the goals of the project and its importance, the theory behind the study, the hypotheses to be tested, and the tests to be used.

The following is a relatively well-written Specific Aims:

> A number of clinical diseases have been associated with disorders of retinal pigment epithelium (RPE) transport and barrier function. The long-term goal of this research is to fully characterize these properties of human RPE to facilitate treatment and perhaps prevention of these diseases.
>
> During the last period we also developed and standardized a new method by which fluid fluxes can be measured directly rather than calculated from isotope fluxes, which are subject to cumulative experimental errors. We plan to incorporate this method into our proposed studies, to test the following **hypotheses:**
>
> a) Cultured fetal human RPE, under normal conditions, transports fluid from its apical side to its basal side utilizing a $Na+$, $K+$, $Cl-$ cotransport system as well as a $Na+$, HCO_3- cotransport system.
>
> b) The activities of these transport systems are modulated by intracellular cAMP concentrations.
>
> c) Cultured fetal human RPE mediated transepithelial fluid movement is modulated by beta adrenergic agonists, histamine, prostaglandin E1, and vasoactive intestinal peptide (VIP) that alter intracellular cAMP concentration. In addition, agents that alter intracellular cAMP metabolism, such as the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), also alter human RPE mediated transepithelial fluid movement.
>
> To test these hypotheses, we propose studies with the following specific aims:
>
> 1. To characterize cultured fetal human RPE transepithelial transport by extending Ussing chamber studies using pharmacologic probes, ion manipulation, and isotope flux studies.
> 2. To determine how cultured fetal human RPE transepithelial transport is modulated by intracellular cAMP.
> 3. To determine the degree to which cultured fetal human RPE transepithelial transport may be regulated by extracellular receptors (such as those to beta adrenergic agents) and to determine the degree to which cultured fetal human RPE transepithelial transport is affected by agents (such as IBMX) that alter intracellular cAMP metabolism.

In the original, this Specific Aims section just filled one page. The hypotheses could be improved by deleting the phrases about methods and emphasizing the hypothesized movement of fluid in real life. The Specific Aims themselves could be improved by eliminating the editorializing, since these comments are repeated in the Methods section. This was for a competing renewal proposal, so reference to past productivity and continuity of work is good.

THE ABSTRACT

The Abstract (called "Description" in PHS 398) should contain (1) the essence of the Specific Aims; (2) a few short sentences concerning the health relatedness of the research; and (3) its scientific significance in terms of its long-term goals. Such statements are often added to the Specific Aims as well. This is useful, provided that the essential parts of the section are not shortened to make room for this addition.

The following is an acceptable abstract in that it expresses a hypothesis and states the experimental approach to its testing. The significance

of the proposed work is also presented. But it fails the appearance test. There are no spaces. Hypothesis, Method, and Significance are not highlighted. It is jammed into the box.

Magnesium (Mg) deficiency may play an important role in the pathogenesis of enhanced vascular reactivity in hypertension. The overall hypothesis to be evaluated is that Mg deficiency caused by glucose intolerance, insulin resistance, or other factors in hypertensives leads to increased vasomotor tone via altered release of vasoactive cycloxygenase and lipoxygenase products of arachidonic acid and enhanced angiotensin II (AII) action. To evaluate the effects of Mg deficiency in normal subjects we will induce the condition by administration of a low Mg diet. Vascular and adrenal sensitivity to AII, platelet aggregation, and eicosanoid levels will be studied prior to and after Mg deficiency is established. Since evidence suggests that Mg deficiency can modulate insulin action, the effect of this deficiency on glucose tolerance will also be studied. In another project the effect of insulin on intracellular Mg levels will be studied using a new fura 2 Mg dye technique. These studies will be performed in groups of subjects with varied blood pressure and insulin levels. Also the effects of acute intravenous and chronic oral Mg loading on the above parameters will be studied in similar subject groups. We will directly study the effect of Mg on AII, insulin, and insulin-like growth factor action in isolated and cultured adrenal glomerulosa cells. Concentration of Mg will be varied and signal transduction and steroidogenic effects will be evaluated. These studies will provide insight into mechanisms important to the pathogenesis of altered vascular reactivity of subjects with hypertension or hyperinsulinemia.

An Abstract that completely fills the box, without line spaces or indentations, affords a repulsive aspect for a tired reviewer, who may well decide to skip it. An Abstract of three or four paragraphs separated by line spaces and containing the words "Hypothesis" and "Specific Aims" in bold, on the other hand, catches the eye, promises interesting informative reading, and has a positive impact on the reviewer (see below).

Magnesium (Mg) deficiency may play an important role in the pathogenesis of enhanced vascular reactivity in hypertension. The overall **HYPOTHESIS** to be evaluated is that Mg deficiency caused by glucose intolerance, insulin resistance, or other factors in hypertensives leads to increased vasomotor tone via altered release of vasoactive cycloxygenase and lipoxygenase products of arachidonic acid and enhanced angiotensin II (AII) action.

Specific Aims: (1) Determine the effects of low Mg on vascular and adrenal sensitivity to AII (platelet aggregation and eicosanoid levels, and glucose tolerance). (2) Determine the effect of insulin on intracellular Mg levels (fura 2 Mg dye technique). These studies will be performed in subjects with varied blood pressure and insulin levels. (3) Determine the effects of acute intravenous and chronic oral Mg loading on the above parameters. (4) Determine the signal transduction and steroidogenic effects of Mg on AII, insulin, and insulin-like growth factor action in isolated and cultured adrenal glomerulosa cells.

Significance. These studies will provide insight into mechanisms important to the pathogenesis of altered vascular reactivity of subjects with hypertension or hyperinsulinemia.