PROTEIN SECRETION

A CRITICAL ANALYSIS OF THE VESICLE MODEL

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Stephen S. Rothman
University of California
San Francisco, California

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Tell all the truth but tell it slant,
Success in circuit lies,
Too bright for our infirm delight
The truth's superb surprise;

As lightning to the children eased
With explanation kind,
The truth must dazzle gradually
Or every man be blind.

EMILY DICKINSON

Preface

Picasso said that the creation of new art forms can come about only as the result of the rejection of older esthetic values. That is, in art, creation is in the first instance an act of rejection or a negative, rather than an affirmative event, contrary to the popular notion of such things. The issue is much the same in science, and new perceptions of nature—discoveries and inventions—come about, or so it seems, most often as the result of questioning older perceptions, and not merely as a continuing positive accumulation of new knowledge, as is so often thought. Indeed, it can be argued that such rejection is an absolute prerequisite to all fundamental shifts in our view of the universe around us.

Rejection often starts modestly in the form of unanswered questions or anomalous observations that current models or paradigms cannot explain in a satisfactory fashion. Sometimes, although certainly not always, as the result of such problems a dominant view of a system is challenged, and evidence is sought not merely in its support but in an attempt to test its predictive power in a questioning manner. Under such circumstances defenders of a paradigm may be constrained to try to make the anomalous fit and to answer the unanswered within the borders of their construct.

This book is about such a situation. It concerns questions that the author and others have raised about the current paradigm for the movement of proteins across biomembranes, and that paradigm's harmony with nature. As I shall discuss in what follows, the paradigm originated in and has been bolstered by a widely held belief that protein molecules pass neither through the substance of biological membranes nor through channels within them. In great part as a result of this belief, models have been developed to explain the transport of proteins by processes that do not require their movement through membranes individually.

It is these models, which we can call "vesicle transport," and the accompanying belief in the essential impermeability of membranes to proteins, that my colleagues and I have questioned. At first our questioning was tentative, but as we explored the issues, testing predictions of the vesicle model as we went, our original questions were not satisfactorily answered, and our uncertainty grew.

As a result, we realized two things. The first was that much of the evidence that had been presented in support of the vesicle paradigm gained its strength as evidence from the a priori assumption that biological membranes were impermeable to proteins. If this support was removed, then the evidence became much more ambiguous and uncertain. The second realization was that if our questions about the paradigm were correct, then we needed to develop an alternative theoretical framework in which to explore protein transport. The obvious alternative, and one that had been in my mind from the beginning, was to apply a traditional diffusion-based construct. Indeed, in my first paper on the subject, regarding protein secretion by the exocrine pancreas, in *Nature* in 1967, I used the term "protein transport," I believe for the first time to describe such processes. This, instead of descriptive terms for particular hypothetical vesicle mechanisms as had become traditional, was an attempt to focus attention on the fact that the secretion of protein should be viewed in the most general manner and not as a subject apart from the study of biological transport otherwise.

In a diffusive model one does not make a priori assumptions about whether a membrane is permeable to a substance, but instead the kinetic and thermodynamic characteristics of the system are experimentally determined and from that knowledge models are developed in an attempt to explain what is observed. If the membrane is permeable to the substance of interest, one starts with simple diffusion as the explanation of parsimony, and rejects it only when and if the evidence requires more complex constructs, and even then, unnecessary complexity is eschewed as we move to the next required level of complexity.

Our particular diffusion-based hypothesis for protein transport, known as the equilibrium theory, has three important characteristics that recommend it as a satisfactory theoretical perspective from which to try to understand the nature of these transport processes. The first is that it is a true alternative to the paradigm. This gives us the power of choice in attempting to account for observations in terms of theoretical models. The second characteristic is that it is readily testable. The third characteristic is that it is a deep or general theory which provides the broadest framework upon which to build an understanding of transport processes, being based, as it is, on general properties of matter.

Applying a diffusive model to protein transport means rejecting the assumption that proteins cannot pass through or otherwise cross membranes individually. It is important to mention that one need only reject the *assumption* that this is true. We need not assume that membranes *are* permeable to proteins by diffusive processes. This is matter for experimental exploration and evidence.

The belief that proteins cannot cross membranes in any kind of general sense was, and is, deep-seated and dates back to the early days of cell theory and our definition of cells themselves. As a result, my colleagues and I encountered much resistance to this idea from others, as well as within ourselves, despite that fact that the evidence that membranes are impermeable to proteins in general was not only not compelling, but totally absent.

In the 20 years since the ideas discussed in this book first saw the light of day, two events may have had an important impact and deserve special note. The first was that the paradigmatic view of protein transport became even more firmly established when a Nobel prize was awarded to George Palade, a scientist whose work and ideas were central to its development. The climate after such an affirmation is less receptive to a different opinion. The second, and somewhat paradoxical, event was the growing realization that individual protein molecules did indeed cross biological membranes, and that this was perhaps a very common occurrence; indeed it was even required by the paradigm itself. I say that this is "paradoxical" because as the vesicle paradigm became more and more firmly ensconced as the manner of means whereby protein transport occurred, its underlying premise, that membranes are impermeable to proteins, was shown to be incorrect even if one attempted to explain such transport solely within paradigmatic borders.

The subject is approached here in great part by considering how cells secrete their protein products. The book has been divided into three sections. The first brief section, *general considerations,* sets the stage for what follows. In it I define terms and present the context in which I will consider the vesicle paradigm. In the second section, *the vesicle paradigm* is described briefly and then considered critically in regard to certain of its aspects, in particular, its underlying assumptions (namely, impermeability), the proposal for filling secretion granules with product, the exocytosis model for secretion, and the signal hypothesis. In the third section, *testing the paradigm,* I discuss a variety of experimental tests of the paradigm, other observations that bear on its adequacy as a general model for secretion and protein transport, and also consider these observations from the viewpoint of an equilibrium or diffusion-based theory of transport. Although I find the general discussion at the beginning helpful for what follows, some readers may wish to go right to the experimental issues.

When I have been faced with a choice between a discussion of experimental details and a more accessible consideration of the question aimed at the nonexpert, I have tried to choose the latter. The primary literature is there to be examined and considered in greater detail for those who wish to do so. I have not written this book solely for those who are actively engaged in studying protein transport, but for a much larger group of people who have an intellectual curiosity about the subject, from biologists working in other areas, to students of biology, and even the curious layman who has the necessary background.

The current paradigm for protein secretion, known variously as the vesicle model, the cisternal packaging–exocytosis model, the exocytosis model, the segregation model, and so forth, is known, not only to professional biologists, but to many college, high school, and, dare I say, grade school students who have been exposed to cell biology. In considering the subject I will assume some knowledge of this model and the character of the evidence that has been adduced in its behalf. The information that I present may not be sufficient in itself if you are not familiar with these ideas, and I suggest that in that case you read one or more of the review articles listed in the bibliography at the end of Chapter 6, which, although often written with different audiences in mind, present the model from a similar perspective that has changed little over the years. These articles have been written by its inventors and should give the reader a sense of how they view its nature, origins, and the evidence in a way that I probably cannot convey as well, or as accurately. However in the final analysis, an informed opinion about the paradigm is not possible without the required search of the primary literature and a thorough and critical reading of the studies that form its experimental basis.

There is one theme in the book that goes beyond the specific subject matter. It has to do with the way in which we go about developing hypotheses, models, constructs, and theories. Although imagination is crucial to the development of hypotheses, it cannot be given free rein in science. This despite the fact that history has taught us that chance, error, and erroneous hypotheses have all played important roles in scientific progress. It seems to me that this is more difficult in biology than in the physical and chemical sciences, because it is generally agreed at the outset that we are dealing with an inordinately complex system. As a result, it often seems reasonable to imagine a complex mechanism at work, and for this reason rather complex models are at times put forth seemingly without sufficient thought to or exploration of alternatives, and evidence is merely sought in their behalf. When positive experimental evidence can be obtained, it affirms the correctness of the model. However, when such results are critically analyzed, they are often found to be weak and uncertain as evidence in support of the investigator's particular dei ex machina, as opposed to other potential explanations. I believe that protein transport, in particular, the secretion of protein by cells, is a case in point.

In a recent lecture at Berkeley, Salvatore Luria, one of the fathers of the great 20th century achievements in molecular biology, made this comment, which I paraphrase: "A scientific hypothesis should never, even tentatively, be accepted as being true, unless it has been exposed to tests that are capable of proving it false." I believe that the particular vesicle theory that I consider in detail here is a hypothesis that has not been subjected to, nor in some cases is even capable of being subjected to, tests that might falsify it. Yet it has not merely been accepted tentatively, as unwise as that might be, but is so widely believed to be true that textbooks at all levels discuss it as established fact. The alternative hypothesis, the equilibrium theory, that I discuss in this book, was in the first instance, and remains at its core, an attempt to develop potential falsifying tests for the vesicle hypothesis. In my view, it is primarily on this basis that its usefulness as a theory should be judged.

Questioning views that are widely held, whatever their factual basis or intellectual justification, is rarely appreciated. If my criticism appears to be tendentious in places, the reader has my apologies. It is my desire to have the issues viewed fairly on their merit, not hyperbole.

Acknowledgments

I dedicate this book to my family, my wife Doreen, and my children Jennifer and Peter, without whose nourishing love and shared experience I could not have overcome the obstacles during these years. I would also like to dedicate it to the memory of my father, Abraham Rothman.

I owe my deepest gratitude to my students and scientific collaborators, who have experienced with me not only the exhilaration of discovery, and the other personal rewards of science, but its dark side as well. "How I wish that somewhere there existed an island for those who are wise and of good will," Einstein importuned.

I would like to thank the many people who have offered encouragement and support, particularly those who gratiously offered to read the book in manuscript form and offered their valuable comments and criticism. I would like to thank Dr. Thomas Ermak for the micrographs in Chapter 4. I would also like to thank Stanley Kudzin and the staff of John Wiley, whose expertise and professionalism have been invaluable.

Stephen S. Rothman

San Francisco, California
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PART 3 TESTING THE PARADIGM

PART 1

General Considerations

Before a theory explaining a certain process can be tested, the process must first be known.

ALBERT EINSTEIN

CHAPTER 1

Two Processes

I will discuss two related biological processes—the secretion of organic products by cells and the transport of one of these products, proteins, across biological membranes. Our understanding of each of these processes has become entwined with that of the other. This intermingling has historical, intellectual, and experimental roots whose basis and character will form an important and recurring theme throughout this book.

1.1 SECRETION

Secretion can be broadly divided into two types. The first is secretion in which substances, mostly fluid and electrolytes, move across polarized tissue surfaces, particularly epithelia, from one extracellular compartment to another, namely, from blood or the internal milieu to bodily cavities or the external milieu. Its opposite is absorption. The second type of secretion is the release or efflux of organic compounds from cells that manufacture or accumulate them.

Only this second type of secretion will concern us here and my use of the term "secretion" will refer exclusively to this process. Such secretion processes are widely seen, diverse in terms of the biological products