

BIOENERGETICA

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**Perspectives on mitochondrial transport
and energy metabolism**

Preface by
Giorgio Lenaz





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*To my wife Ersilia
and to my nephew Riccardo
who might be reading this book.*

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Preface

At first glance, this appears to be a specialized book on mitochondrial transport and carriers, and therefore a book written to be read by bioenergeticists and researchers working on metabolite transport. This is not really the case. The book will certainly be enjoyed by bioenergeticists - indeed I myself read it with great interest. However, the book is really written for students who are tackling biochemistry for the first time in their courses. It aims to teach them to subject to critical reasoning the material with which they are presented. In other words, its aim is teaching the students how to **learn** rather than just absorb and remember information.

Given that as the true purpose of the book, why does it focus on mitochondrial transport? Professor Passarella has worked for all of his career on this subject,⁷ and therefore, he knows it intimately, the pertinent literature and the controversial aspects. Using this as a resource, he teaches the student how to search for publications in the literature on a given set of subjects using PubMed and other search engines, and accompanies the student in the analysis of key papers, comparing different results and explaining the reasons behind the controversy. Through this approach, the Author makes the reader aware that the reason for controversy is often to be found in mere ignorance and misunderstandings of previous papers. With rigorous reasoning, Passarella leads the reader to the correct answer and to an understanding of the molecular logic at the basis of such a correct answer. Hence, the student is not considered to be a passive container of information, but an active player, who will eventually acquire the capability of deducing by himself the correct answers by a critical examination of the experimental findings.

In all of these discussions, the Author convinces the reader by a forensic analysis of experimental data in the pertinent literature. When tackling any subject, the student has to ask a series of questions related to a given process. These deal with the quality, quantity, location, possibility of change, the nature of change and the rate of such a change, and finally why such process exists.

The first two chapters of this book are particularly interesting. They lead the student to the fundamentals of enzymology and to the methodology used to study transport. It is clear that in this section, the wish of the Author is to make the student understand the basic concepts. The Michaelis-Menten equation is not derived as a pure mathematical exercise, it is clarified in its most intimate significance, so that the student really understands what is all about!

In the next chapters, Passarella examines some important transport systems of the inner mitochondrial membrane. For the reasons outlined above, even some notions accepted in the textbooks as solid facts are challenged and are demonstrated to be incorrect. You can go through the book and will find all kinds of commonplaces reported by textbooks but wrong.

Typical is the notion of oxaloacetate transport. All textbooks passively accept the idea that there is no mitochondrial carrier for oxaloacetate, despite the experimental evidence, as already shown by Haslam and Krebs in 1968. It was later shown by the author of this book that there are in fact three carriers in the inner mitochondrial membrane mediating exchange of this molecule with pyruvate, L-lactate and malate respectively. The pyruvate/oxaloacetate and the L-lactate/oxaloacetate antiporters are important in gluconeogenesis and the malate/oxaloacetate antiporter plays a major role in the shuttle for the re-oxidation of glycolytic NADH. A forth carrier for oxaloacetate is the antiporter D-lactate/oxaloacetate. Similarly, the common notion concerning the fate of fumarate produced by the argininosuccinate lyase reaction in the urea cycle is that fumarate is hydrated to malate in the cytosol, then malate is carried into mitochondria and converted back to aspartate. However, this notion is false, since there is no fumarase in the cytosol. The truth is that there are two transporters in the inner membrane carrying fumarate inside to the matrix.

Another fact ignored in Biochemistry textbooks is that L-lactate influx occurs in mitochondria where the compound may be metabolized undergoing NAD-linked oxidation. A similar observation concerns mitochondrial phosphoenolpyruvate influx and the existence of a mitochondrial pyruvate kinase: again, you will not be able to find this notion in any textbook.

In some instances, the amount of knowledge required for an inexperienced medical student to follow the subtle reasoning of the author seems excessive, and may make the book rather daunting, but undoubtedly a clear message is conveyed in all chapters of this book. This message is that the student does not have to passively accept whatever he reads in textbooks, but has to use a critical mind and be ready to accept changes and even drastic corrections

coming from new experimental evidence. Now this is what we know. Later it could be different!

Furthermore, this book teaches the students, and reminds us, how fascinating is Biochemistry, a modern science that is never static but always evolving. Perhaps this book will inspire some of the students reading it with their fresh minds, prepared and free of prejudice, to become part of the next generation of scientists working creatively in the field of biochemistry and molecular sciences.

Giorgio Lenaz
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Introductory remarks

Our ever-increasing knowledge of biology, physics and chemistry provides the fundamental underpinning to the practice of medicine. The biochemistry courses of a good medical curriculum should be designed not only to give the students a thorough knowledge of that science base, but also to make them aware that because this base knowledge is continually changing, they will need to develop this knowledge and its application during their professional career.

I hope that with this book the students will be encouraged to adopt an approach to learning based on curiosity and the exploration of knowledge rather than on its passive acquisition, because it is important for them to develop this ability to apply new knowledge, and to adapt to changing circumstances in their professional life.

By means of this book, I will also help students to develop their intellectual skills, such as the understanding and interpretation of scientific evidence, problem solving skills; skills which can be transferred to further stages of their medical education.

This book does not substitute exhaustive texts of biochemistry, but instead aims at preparing the twenty-first century students' state of mind. The pages of this book are meant to offer formation, rather than information to readers who will analyse in detail any aspect of the reported "biochemistry", thus aiming at encouraging critical thinking with no leaving gaps due to unresolved queries. However, with this book I would like to warn the students against passive acceptance of all the information that both the textbooks and important journal can offer: any student should be able to verify the truth of the science by taking advantage of the variety of the available data base.

Once defined the starting point and the destination, any learning process employs strict yet simple logic to which must not be opposed, in the name of a student's freedom, a singular method of learning which may sometimes be superficial due to laziness or lack of zeal, at times overly complicated due to excessive details, often obtuse and presumptuous.

Remember that what is written in italics was written with the aim of accompanying the reader in the understanding the issues reported in the above lines and helping the readers in elaborating upon it. The italics establish a sort of friendly and didactic scientific dialog between author and reader.

S.P.

Bari, January, 2019

Instructions for readers

How do I practice and enjoy biochemistry on my own?

When studying Latin I was interested in a Cicero's paper entitled "De finibus bonorum et malorum" in which Good and Bad were treated according to stoic and epicurean philosophies. Cicero warned that before talking about Good and Bad it is necessary to define what Good and Bad are. Thus, I learned that in order to talk about something, we must know what you are talking about. In particular, in studying Biochemistry you must be able to define anything considering both the chemical structure and the biological function/s. In this regard any dictionary, perhaps an etymological dictionary can be used. Note that most scientific terms are derived from Greek and Latin; moreover, to have exhaustive knowledge of any subject, according to what Greek and Latin rhetoricians taught, you must answer a number of questions including;

- **of whom or what it is?**
- **to whom is it directed?**
- **for what reason(s) it occurs?**
- **where it occurs?**
- **from where it derives?**
- **where is it directed?**
- **must it go across something?**
- **how it occurs?**
- **what is its goal?**
- **when it occurs?**
- **as a result of what physiological need it occurs?**

Indeed, the five Ws (Who, What, Where, When, Why) rule is not an original product of English/American linguists: the rhetorician Hermagoras of Temnos (200 b.C.), defined seven "circumstances" as the loci of an issue:

Quis, quid, quando, ubi, cur, quem ad modum, quibus adminiculis.

(Who, what, when, where, why, in what way, by what means).

Sometimes, "nihil sub sole novum!" Nothing new under the sun!

Moreover, you must be capable of taking advantage of and using a variety of adjectives which will add special information to the subject you are dealing with. Notice that the presence of an adjective can itself suggest that there are different manners in which the same process can occur. For instance, if you are talking about "oxidative phosphorylation", having established that phosphorylation is the addition of inorganic phosphate to ADP to produce ATP, the term "oxidative", besides suggesting that this ATP synthesis is due to oxidation processes, postulates that ATP synthesis can also occur in different manner. (i.e. substrate-level and photophosphorylation in which high

energy substrate and light (φως in Greek) are used to provide energy needed for ATP synthesis, respectively.

Often to try to understand what a word itself suggests is useful exercise to learn. Consider the word “gluconeogenesis”, I suppose that is easy enough to consider this word as deriving from three word:

Gluko= glucose, neo = new, genesis=origin, creation, birth. Thus, gluconeogenesis is a process in which glucose is created

- for what reason(s) it occurs? If the cell synthesises a molecule, it is evident that the organism needs such a molecule (remember the Le Chatelier-Brown principle).
- What is its goal? Simply to give glucose. Glucose is an energy source, however other molecules can be used as energy source, including lipids and proteins...therefore is obvious that glucose synthesis is required for cells that cannot use energy sources different from glucose. This could be also the answer to the question: as a result of what physiological need it occurs?
- When it occurs? When glucose is not enough, when it has already been consumed, likely some hours after the meal, understarvation. However, it seems probable that we need always glucose, but why? Is/are there an organ/some cells which is/are always working which cannot use other compounds? Perhaps the brain and erythrocytes!?
- In which manner it occurs? Certainly, in a manner in which energy is used: any anabolism/synthesis requires precursors and energy.
- Where it occurs? Obviously, gluconeogenesis could occur in any cell which needs glucose, but, according to “underlying principle of molecular economy in living organisms” in the terms of Albert Lehninger i.e. the principle of maximum economy of parts and processes, it should be better that the main glucose producer is one organ from which the newly synthesized glucose can get its final destinations. Probably liver could be the proper organ devoted to gluconeogenesis in our body. Since the hepatocyte has different cell components, could we predict where in the cell gluconeogenesis takes place? Since energy is required for any anabolism, since mitochondria are the cell powerhouse then it is probable that mitochondria play a role in gluconeogenesis, however given that glucose must be exported in the blood stream to get its final destination, it seems probable that glucose synthesis occurs in the cytosol. On the other hand, since glucose is catalysed in glycolysis, in the light of the principle of maximum economy of parts and processes, it would be a good thing to use some enzymes of glycolysis able to catalyse reversible reactions in the cell.