Preface

The idea of writing a book on the analytical methods for the study of carbohydrates, essential components of the central metabolism of land plants, unicellular green algae, and cyanobacteria, came to me at the insistent request of my best student who insisted that I should pass on the experience I had gained over so many years devoted to this field.

At first, the request seemed simple to implement; however, I had to tell my experience in a way that no other published texts had done so before, where general principles of separation and purification of proteins or methods to quantify a given carbohydrate were provided. It suddenly occurred to me that the best way to achieve this goal was to describe the different methodologies that I had been extensively using in my laboratory as if I were orally explaining them to my students or colleagues who were not familiar with them. My intention was, to some extent, to show that carbohydrates and their metabolic pathways could be studied by following quick and easily accessible methodologies.

Building on my idea, I decided to describe in detail each experiment to be performed, starting from the technique's fundamentals or principles, mentioning the necessary steps for implementation, also including the biological starting material, and the choice of methodology among different alternatives. To put it into a few words, I intended to provide researchers with tools and information ranging from an overview of the extraction and purification of the enzymes involved in carbohydrate metabolism from photosynthetic organisms to different analytical techniques for the measurement of their activities, and separation and determination of sugars and other compounds related to them. To me, the clearest way to transmit this information was to apply it to the study of specific cases, selecting the key sugar players. I trust readers will find this approach useful and that it will assist those who are taking their first steps into the field of sugar metabolism.

Perhaps the most important aspect of this book, and what distinguishes it from the literature available, is that it brings to the present a selection of reliable and tested methods used decades ago but not widely applied these days, which do not require costly instruments and specially trained personnel, together with some more modern techniques.

Regarding the book organization, it has been divided into three parts for an orderly and systematic description of the general analytical methods. Part I (see Chapter 1: Determination of Carbohydrates Metabolism Molecules) focuses on procedures for determining the most

relevant carbohydrates and some compounds related to their metabolism, including colorimetric, spectrophotometric, and spectrofluorimetric methods. The applications of these methods are presented in later chapters. Part II starts with the description of general methods for the extraction of proteins with enzymatic activity involved in sugar metabolism (see Chapter 2: Preparation of Protein Extracts). Chapter 3, Protein and Carbohydrate Separation and Purification describes the most common methods for separating proteins (ion exchange, gel filtration, affinity chromatographic methods, isoelectrofocusing, and fast protein liquid chromatography) and carbohydrate fractionation (chromatographic methods, including HPLC (high-performance liquid chromatography) and HPLC coupled to mass spectrometry). The determinations of enzyme activities, measured as either substrate consumption or as product appearance, are reported in Chapter 4, Measurement of Enzyme Activity. The next chapter deals with a general overview of the fundamentals of the methodologies based on mass spectrometry (MS) and nuclear magnetic resonance (NMR), which despite not being laboratory bench methodologies are sometimes resorted to through external services. Specific cases are covered in Part III, starting with sucrose, the main plant sugar (see Chapter 6: Case Study: Sucrose), and following with trehalose (see Chapter 7: Case Study: Trehalose), raffinose (see Chapter 8: Case Study: Raffinose), fructose polymers or fructans (see Chapter 9: Case Study: Fructans), polysaccharides in general (see Chapter 10: Case Study: Polysaccharides), starch (see Chapter 11: Case Study: Starch), and glycogen (see Chapter 12: Case Study: Glycogen), reserve polysaccharides of plants and cyanobacteria, respectively, and structural polysaccharides as cellulose (see Chapter 13: Case Study: Cellulose). This third part ends with the description of the main sugar-phosphates (see Chapter 14: Case Study: Sugar Phosphates) and nucleoside diphosphate-sugars (see Chapter 15: Case Study: Nucleotide Sugars) involved in carbohydrate metabolism in photosynthetic organisms.

To close, my deepest gratitude to my wife, Graciela Salerno, who has been of vital support throughout the months preceding the production of the book, and also of fundamental help with the editing and proofreading of the manuscript. Without her help this book would have not been possible. And to my daughter, Sheila Pontis, who has helped me throughout the writing of this manuscript with her knowledge of English, design, and communication. In addition, I would like also to express my sincere thanks to Alejandro Parise for his advice and council on the writing of Chapter 5, General Introduction to Mass Spectrometry and Nuclear Magnetic Resonance, and my special gratitude to Valentina Mariscotti for helping me with the translation, and to Gonzalo Caló and Cintia Pereyra who assisted me toward its completion.

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