Biological pigmentation is a vast topic, and the enormous diversity that has emerged through the process of evolution is astounding. Even if the scope of enquiry is limited to vertebrate melanin pigmentation, there is still a profusion of complex interactions that have hitherto hampered the attainment of a ‘Standard Model’ of melanogenesis.

Melanins and Melanosomes is a compendium of thirteen chapters by experts in the relevant fields, encompassing vertebrate melanin pigmentation from the biology of melanocytes, the biogenesis of melanosomes, and their regulation, distribution, and transfer, the process of melanogenesis, the nature and properties of melamins, and their biological and clinical significance.

Edited by Jan Borovanský and Patrick Riley, this book is intended as an encyclopaedia of current knowledge of vertebrate melanin and its formation. With its discussion of the latest ideas and advances it is essential reading for students, academics, researchers, and clinicians, as well as being a valuable reference volume for professional pigmentologists.

Jan Borovanský (left) was born in Prague in 1943. He graduated from the Faculty of General Medicine at Charles University in 1966, gained his PhD under Prof. J. Duchon in 1976 and was appointed Full Professor of Biochemistry in 2004. He received the Annual Award of the Czechoslovak Oncological Society in 1975 and was Honorary Research Fellow at University College London in 1980 and again in 1984. In 1981 he was General Secretary of the 3rd European Workshop on Melanin Pigmentation and in 1998 President of the 8th Meeting of the ESPCR. Jan Borovanský was a member of the ESPCR Council from 1990 until 1998 and was elected an honorary member in 2010. As a member of the organizing committee of the 14th FERS Meeting in 2009 he was responsible for arranging the symposium on ‘Melanins and Melanogenesis’ from which this book stems. His research has focused on biochemical studies of melanosomes, metal binding by melamins and the cytotoxicity of zinc.

Born in Paris in 1935, Patrick Riley (right) graduated in medicine from University College Hospital Medical School in 1960. He joined Claude Rimington’s department and began a series of cellular studies culminating in his appointment to a chair of cell pathology at UCL in 1984. He was the recipient of the Myron Gordon Award in 1993 and the Centenary Medal of Charles University. Patrick Riley was a founding member of the European Society for Pigment Cell Research and the International Federation of Pigment Cell Societies, as well as organizer and chairman of the XVth International Pigment Cell Conference. He was a founding editor of Melanoma Research, is on the editorial boards of several journals and has published extensively on melanocytes, melanogenesis and melanoma. His work has covered many fundamental aspects of cell pathology, including free radical pathology, cell size control, regulation of cell proliferation, and cancer.
Edited by
Jan Borovanský and Patrick A. Riley

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Melanins and Melanosomes

Biosynthesis, Biogenesis, Physiological, and Pathological Functions
Dedication

“All nature is but art, unknown to thee;
All chance, direction which thou canst not see”

Alexander Pope: An Essay on Man

Were we to ascribe to chance the existence of this volume we should have to begin with the moment in July 1952 when Professor A.F. Richter, Head of the Second Institute of Medical Chemistry at Charles University in Prague, opened a dust-covered cabinet from which he took, apparently at random, a bottle containing a dark powder and handed it to a young assistant with the words: “Young man, study the contents of this flask.” The assistant was Jiri Duchon and the label on the flask read: “Human melanosarcoma, prepared by H. Waelsch.”

Jiri Duchon was born on 27 July 1927, the only son of an eminent scientist. On graduating in medicine in 1952 he joined Richter’s laboratory and his careful analysis of the sample given to him set him upon the course of studies that were to occupy him for the rest of his life. He defended his PhD thesis in 1962 on the topic of “Urinary melanogens in melanoma” and he subsequently made many important contributions to quantitative analysis of the products of melanogenesis. In recognition of his early work, Jiri Duchon was awarded a Roosevelt Fellowship that enabled him to spend 15 months at Harvard in the laboratory of T.B. Fitzpatrick. This was in 1967–1968 when he met and established a friendship with Makoto Seiji who had just developed the methods for melanosome isolation. On his return to Prague, Jiri Duchon set about improving the isolation technique and analyzing these newly discovered organelles. Under his direction and inspiration the Prague laboratory became the leading European center for the detailed biochemical investigation of melanosomes. Jiri Duchon was Head of the Institute for 26 years and many of his collaborators have continued to contribute significantly to the field of study that he promoted.

Professor Duchon was an internationally recognized and highly respected member of the pigment cell fraternity, and was elected an Honorary Member of the European Society for Pigment Cell Research in 1998. It was partly in his honor that the scientific session on “Melanin and Melanosomes” was arranged at the Federation of European Biochemical Societies (FEBS) Congress in 2009, but
tragically he was taken ill on the very day of the Symposium. He was full of encouragement for the project that grew out of the meeting, namely that of publishing a definitive volume devoted to the subject of his academic endeavors, but died on 2 November 2009, long before it was completed.

In recognition of his seminal role in the events that led to the production of this book we dedicate this volume to Jiri Duchon with affectionate remembrance of a fine scientist, an inspirational teacher, a kindly and cultivated companion, and a true friend.

Professor Jiri Duchon MD, PhD, DrSc (1927–2009)
(Photograph by K. Meister)
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Preface

“To that small part of ignorance that we arrange and classify we give the name knowledge”

Ambrose Bierce

This book is entitled *Melanin and Melanosomes*, and is about pigment and pigmentation. It is important, however, that we bear in mind that, while the primary function of melanocytes is the production of pigment in melanosomes, these cells have other attributes and perform other significant functions. Some of these are well recognized, such as the involvement of the retinal pigment epithelium in photoreceptor physiology (detailed in Chapter 7). Another interesting possibility is that melanogenesis may be the source of some of the substrate for dopamine synthesis [1], and melanocytes may have other important neuroendocrine functions as pro-propriomelanocortin processing cells and a source of prostaglandin D synthase (reviewed by Takeda et al. [2]). Some of these actions may go some way to explaining the remarkable anatomical distribution of melanocytes, often in locations that are not illuminated, such as the leptomeninges.

However, this volume is devoted to melanin and melanosomes, and is primarily concerned with vertebrate, especially human, pigmentation. We include melanin that is formed by oxidative processes that are enzymatically catalyzed in specialized cells, both the neural crest-derived dendritic melanocytes (named “classical” melanocytes in Chapter 2) and optic cup-derived retinal pigment epithelial cells (“nonclassical” melanocytes), as well as melanin generated by other oxidative pathways, such as the neuromelanin of the midbrain. The importance of this latter pigment, particularly in relation to Parkinson’s disease, is set out in Chapter 8.

The enzymatically generated melanin in vertebrates is synthesized and deposited in specialized intracellular organelles, the melanosomes, and this book concentrates on the many aspects of the formation and functions of these organelles.

The melanosome is a highly specialized organelle, the history of which owes much to the early work at Charles University under the aegis of Jiri Duchon (1927–2009), to whom this book is dedicated.

Many of the important properties of melanosomes were established in Prague in the early 1970s by a series of investigations on isolated and purified preparations of this organelle, and investigators at Charles University have continued to
contribute significantly to the advancement of this field, and to follow the developments that have taken place in elucidating the structure of melanosomes and the complex biological roles in which they are implicated.

This volume grew from a combination of auspicious factors. In 2009, the 34th Congress of the Federation of European Biochemical Societies (FEBS) was organized in Prague. Naturally, with one of us (J.B.) on the Organizing Committee, one of the scientific sessions was devoted to melanosomes and, in the wake of the discussions at this meeting, it was felt that there was a significant body of new data relating to melanosomes that could usefully be assembled in a volume devoted exclusively to this organelle.

It was hoped that such an overview, by integrating the diverse aspects of current knowledge, might help to generate a new understanding of the biological role of melanosomes and stimulate novel research effort in this interesting area of study.

We have been fortunate in our publisher, Wiley-VCH, who recognized the timely nature of the proposed volume, and we thank our commissioning editor, Gregor Cicchetti, and his team, especially Anne Chassin du Guerny, for their help and encouragement in bringing the project to fruition.

Of course, our main thanks go to our panel of distinguished international contributors who have generously given of their time and expertise in preparing the chapters that we hope form a coherent picture of the up-to-date knowledge in the field.

Last, but not least, this book celebrates a long and fruitful collaboration between the Editors involving many visits between Charles University and University College London. It is a pleasure to acknowledge the assistance of the British Council in enabling these exchanges.

We had hoped initially to have the opportunity to arrange the order of the chapters in the light of their ultimate content so that overlapping areas were most rationally ordered to enable the volume to be read more or less in sequence while allowing the, perforce abundant, cross-references to act as a secondary web in a cohesive network. However, pressure of time prevented us from completing this task and readers may find it more convenient to skip between the various contributions according to their interests and predilections. In principle, although the topics are inextricably intertwined, we have elected to place the contributions devoted to melanin—its biosynthesis, chemistry, and properties—at the front of the book, and those dealing with melanosomes—their structure, biogenesis, distribution, and properties—in the following chapters.

The topic is put into chronological context by a historical Introduction in Chapter 1, in which Jan Borovanský traces the steps in the discovery of the melanosome, illustrated by portraits of the important investigators that took part in these exciting early studies.

As this book is directed largely at aspects of human pigmentation, Chapter 2 consists of a detailed overview by Sophie Colombo, Irina Berlin, Véronique Delmas, and Lionel Larue of the specialized cells in vertebrates in which melanin production in melanosomes takes place. In their contribution a distinction is made between “classical” and “nonclassical” melanocytes. Chapter 3, by Patrick Riley,
Christopher Ramsden, and Edward Land, emphasizes the central role of the generation and reactivity of o-quinones in melanogenesis, and is followed by Chapter 4 in which the biosynthesis of melanins is reviewed by José Carlos García-Borrón and Conchita Olivares Sánchez. Chapter 5, by Alain Taïeb, Muriel Cario-André, Stefânia Briganti, and Mauro Picardo, comprises an analysis of inhibitors and enhancers of melanogenesis. The current understanding of the structure of melanins is then reviewed in Chapter 6 by Shosuke Ito, Kasumasa Wakamatsu, Marco d’Ischia, Alessandra Napolitano, and Alessandro Pezzella, and this is followed in Chapter 7 by a description of the properties and functions of ocular melanins and melanosomes by Małgorzata Rózanowska. Chapter 8, by Kay Double, Wakako Maruyama, Makako Naoi, Manfred Gerlach, and Peter Riederer, is devoted to the biological role of neuromelanin in the human brain and its importance in Parkinson’s disease. Chapter 9 consists of a detailed review of the biogenesis of melanosomes by Cédric Delevoye, Francesca Giordano, Michael Marks, and Graça Raposo. This is followed in Chapter 10, by Mireille Van Gele and Jo Lambert, by a description of the transport and distribution of melanosomes. The genetics of melanosome structure and function are skillfully summarized in Chapter 11 by Vincent Hearing. Chapter 12, by Jan Borovanský and Patrick Riley, is devoted to the properties and functions of melanosomes, and, in Chapter 13, the abnormalities of melanosomes and melanogenesis in melanoma precursor lesions are discussed by Stan Pavel, Nico Smit, and Karel Pizinger.

We firmly believe that this compilation of expertise embodies a significant work of scholarship, and we sincerely hope that the combined wisdom embraced by this volume conveys both the breadth of detailed and exciting knowledge that currently exists about melanin and melanosomes, and also reveals those shadowed areas of doubt and ignorance that await illumination in the future.

March 2011

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History of Melanosome Research

Jan Borovanský

1.1

Introduction

Melanosomes were first proposed as specific organelles, unique to pigment cells, in a preliminary publication that appeared on 30 July 1960 [1]. An announcement had been made at the 21st Annual Meeting of the Society for Investigative Dermatology, at Miami Beach, Florida, USA on 13 June 1960 [2] and the news, that the chemical composition and enzyme activities in melanosomes and mitochondria are completely different, was considered to be of such significance that it appeared in a newspaper report (Figure 1.1). Similar data, with an emphasis on terminology, were published in 1963 [3].

This advance was the result of collaborative work between M. Seiji (1926–1982), at that time working at the Department of Dermatology, Harvard Medical School in Boston under the leadership of T.B. Fitzpatrick (1919–2003) (Figure 1.2), and H. Blaschko and M.S.C. Birbeck, with whom Dr Fitzpatrick established scientific cooperation during his tenure of a Commonwealth Fellowship at the Department of Biochemistry, Radcliffe Infirmary in Oxford.

The history of melanosome research can be formally divided into three parts: (i) the pre-Seiji era (prior to 1960), (ii) the Seiji era (1960–1982), and (iii) the post-Seiji era (1983–).

1.2

Melanosome Research in the Pre-Seiji Era

The first description of mammalian pigment cells was published by Gustav Simon in 1841 [4] who observed round and stellate pigment cells in the hair bulbs of pig embryos. It was preceded in 1838 by Purkyne’s description of pigment in the cells of the substantia nigra, which not only drew attention to pigment granules, but also noted the rise in their numbers with age [5]. We have to admire these early reports because their authors, armed only with primitive light microscopes, were able to ascertain that melanin was not diffusely distributed in the cytoplasm...
of pigmented cells, but was present in the form of discrete aggregates [5, 6] (Figures 1.3 and 1.4).

Deciphering the old literature is problematical as authors often fail to distinguish between melanin (the pigment itself), melanoprotein (the natural melanin–protein complex), and melanin granules (the subcellular organelle). If the method of separation is not adequately described, it is difficult to be certain what material was studied and any conclusions can be misleading [8]. The lack of electron microscopic identification of isolated material led to many misinterpretations; for example, the “melanopseudoglobulin” studied by Greenstein et al. [9] was later shown to be melanosomes [10] and Bolt’s “melanoprotein” [11], widely used in biophysical studies, turned out to consist of damaged melanosomes [12]. Mason et al. [10] posed the question of whether melanin granules were particles with a specific structure or consisted of random aggregates of precipitated metabolic
products. The introduction of electron microscopy was able to resolve this matter and Laxer et al. [13] were able to discern an inner ultrastructure in isolated melanosomes. The first clear pictures were obtained only in 1956 [14].

An avalanche of papers in subsequent years brought with it enormous amounts of information on the ultrastructure of melanosomes and its changes during
melanosomal development (good examples are [15–17]). Other papers (reviewed in [18]) brought together ultrastructural and biochemical data that, in combination, laid the basis for the nomenclature of melanosomal ontogenesis.

By comparison with the morphological data, biochemical investigations of melanosomes were more modest, mainly due to the fact that ultrastructural data were derived from studies of intact cells or tissues, whereas biochemical research used samples prepared by relatively harsh preparative procedures. These samples sometimes consisted of melamins, or altered melanosomes, or their fragments, usually without any check of their nature or homogeneity [18].

The aim of researchers in the nineteenth century was not to prepare subcellular particles or native melanoproteins, but to separate the colored pigment (“Farbstoff” = melanin in the terminology of that time). The presence of protein in the isolated material was considered an unwanted contaminant [19]. Probably the first mild separation protocol was used by J.J. Berzelius [20]. He investigated pigment (melanosomes?) obtained from eye membranes by water extraction, and noticed its insolubility in acids and limited solubility in alkali. Similar mild extraction procedures were used by Landolt [21] and Mörner [22]. The early isolation procedures were reviewed by Waelsch [23]. He studied “natural melanin” from human melanoma metastases and horse choroids, confirmed the presence of protein attached to pigment, and suggested that melanin could be synthesized from the cyclic amino acids present in the protein moiety; this idea has not been
abandoned till now. Herrmann and Boss [24] demonstrated dopa oxidase activity in the fraction of melanin granules from ciliary bodies of cattle eyes, but, as their samples were contaminated with mitochondria, they demonstrated the presence of mitochondrial enzyme markers as well. In 1949, du Buy et al. concluded that melanosomes are modified mitochondria typical of pigment cells [25]. It is interesting that du Buy [26] and other authors [27] did not abandon the mitochondrial theory of melanosome origin even in 1963 (i.e., 2 years after the formulation of Seiji’s melanosomal concept) and even published their papers in the same volume in which Seiji et al. published detailed confirmation of their model [28].

It is interesting that history has disregarded the contribution of Stein [29] who, several years before the work of Seiji et al., using a separation procedure of his own, isolated melanin granules from ox choroids and analyzed their content not only of melanin, but also lipids, carbohydrates, RNA, and metals (including the pioneer finding of a high level of zinc), and concluded that the chemical composition of melanin granules is completely different from mitochondria.

The ability of melanin in melanin granules, isolated from Harding-Passey melanoma and from the ink sac of Loligo opalescens, to act as a cation exchanger [30], and the demonstration of free radical activity in melanin-containing tissues [31] also rank among the observations of the pre-Seiji era.

1.3 Melanosome Research in the Seiji Era

1.3.1 Terminology of Melanosomes

The demonstration of melanosomes as unique pigment cell organelles possessing developmental stages prompted the introduction of a system of terminology that reflected the characteristics of the various states. Until 1961 the common term for all varieties of these organelles was melanin (or pigment) granule [1, 2]. The first system of nomenclature [2] described three stages in the ontogenesis of melanosomes:

i) Premelanosomes: spherical organelles.
ii) Melanosomes: organelles with an internal structure and tyrosinase activity.
iii) Melanin granules: melanoprotein polymer.

A second terminological system was proposed [3, 26] consisting of three developmental stages plus a final product. Thus:

- Stage I (first stage): biosynthesis of protein.
- Stage II (intermediate stage): biosynthesis of organelle.
- Stage III (late phase): biosynthesis of melanin.
- Final product: melanin granule.
These nomenclature systems introduced a certain degree of confusion, particularly as the term melanin granule had been used to describe pigment granules at any developmental stage. In an attempt to establish a consensus, Fitzpatrick et al. [32, 33] circulated a postal questionnaire seeking opinions about the adequacy of the terms in common use in pigment cell research and, with the approval of the participants of the Sixth International Pigment Cell Conference in 1965 in Sofia, Bulgaria, recommended the use of two terms:

- **Melanosome**: a discrete melanin-containing organelle in which melanization is complete as indicated by its almost uniform density by electron microscopy and the absence of demonstrable tyrosinase activity.

- **Premelanosome**: a term applied to all the stages in melanosome biogenesis that precede the fully developed state. Within the restrictions of this general definition, the premelanosomal stage might, at the discretion of the investigator, be subdivided into early, intermediate, and late phases.

The nomenclature in general use today does not adhere to any of the three systems outlined above, but is essentially a system proposed by Toda et al. [34–36] reflecting the earlier descriptions of Birbeck [37, 38] which employs the uniform term “melanosome” with a numerical indication (I–IV) of the degree its ontogenetic development.

However, in practice, chaos prevails. While the system of Toda et al. is widely—if somewhat erratically—used, some European authors refer, often incorrectly, to the stages proposed in the second system of nomenclature [3, 26] and some American authors tend to cite nomenclature introduced in their previous papers or those of their friends.

1.3.2 **Ultrastructural and Histochemical Studies**

The concept of subcellular biosynthesis and localization of melanins and melanoproteins in melanosomes was further confirmed by (i) autoradiographic evidence with $[^3]H$dopa and $[2-^{14}C]$dopa [39–43], (ii) incorporation of $[2-^{14}C]$dopa and monitoring radioactivity in subcellular fractions [44, 45], and (c) isolation of melanosomes and analysis of their chemical composition [46, 47].

Electron microscopy enabled the definition of the basic morphometric data of isolated melanosomes (i.e., their size, shape, and ultrastructural appearance). The most extensive data were published by Hach et al. [48, 49]. For discussion concerning the ultrastructural appearances of melanosomes, see Section 12.3 in Chapter 12.

Various pathological states may be manifested by changes in melanosome morphology. Mishima et al. [50] considered that melanosome polymorphism, such as changes in size, shape, ultrastructural matrix, the manner of melanin deposition, and the degree of melanosome maturation, as a criterion of molecular pathology that could find practical use in the differential diagnosis of various pigmentary disorders.