PIGMENT CELL RES 16: 280–286. 2003
Printed in UK—all rights reserved

Copyright © Blackwell Munksgaard 2003
ISSN 0893-5785

Melanosome Degradation: Fact or Fiction

JAN BOROVANSKÝ[1] and MILAN ELLEDER[2]

[1]Department of Biochemistry and Experimental Oncology and [2]Institute of Inherited Metabolic Diseases, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
**Address reprint requests to Jan Borovanský, M.D., Ph.D., Department of Biochemistry and Experimental Oncology, 1st Faculty of Medicine, Charles University, U nemocnice 5, 128 53 Prague 2, Czech Republic. E-mail: jborov@lf1.cuni.cz*

Received 24 January 2003; in final form 21 February 2003

Our mini review summarizes what is known about the (bio)degradation of melanosomes. Unlike melanosome biogenesis where our knowledge enables us to explain it in molecular terms posing many interesting questions on the relation between lysosomes and melanosomes, melanosome degradation has remained 'terra incognita'. Observations at optical and ultrastructural levels describe the disintegration of melanosomes in the lysosomal compartment (in auto- and heterophagosomes). Histochemical studies suggest the participation of acid hydrolases in the process of melanosome degradation. Biochemical data confirm the ability of lysosomal hydrolases to degrade melanosome constituents except the melanin moiety. The similarity of melanin structure to that of polycyclic aromatic hydrocarbons suggests that melanin should be sensitive mainly, if not exclusively, to oxidative breakdown. In vitro melanin can indeed be decomposed by an oxidative attack and the degradation is accompanied by fluorescence and decreasing absorbance. From enzymes engaged in the biotransformation of polycyclic hydrocarbons only phagosomal NADPH oxidase meets the criteria (particularly as for compartmental and catalytic properties) to be involved in melanin biodegradation. The in vivo biodegradation of melanin has so far been clearly demonstrated in *Aspergillus* and fungi melanins.

Key words: Melanosome, Melanin, Biodegradation, Disintegration, Phagosome, Oxidative degradation

INTRODUCTION

Unlike melanin and melanosome biogenesis, where our knowledge begins to be comprehensive enough to understand these processes and to describe them at the molecular level (1–4), the data available for melanin and melanosome (bio)degradation are limited and ambiguous.

To describe these events: (a) It is necessary to distinguish strictly between the terms disintegration and degradation. In this paper, disintegration means fragmentation into smaller units (a quantitative change), whereas degradation includes chemical destruction of the original structure(s) by converting it/them into metabolites and degradatory products (a qualitative change). In the literature these two terms have been used synonymously which often makes the interpretation of older results troublesome. (b) It is important to keep in mind that the melanosome is a subcellular organelle of higher order consisting of many constituents (5, 6). Melanin represents the most characteristic and often the major moiety but only one from many (see Fig. 1). The description of melanosome degradation would not be complete, if we concentrated only on the fate of melanin.

Melanosome Constituents can be Degraded Hydrolytically Except the Melanin Moiety

The disintegration of melanosomes is a progressive process. Mildly disintegrated pigment granules appear frayed at the edges with melanin fragments nearby. With further disintegration, the melanosomes appear disrupted and their density decreases. In advanced stages the underlying matrix structures may become evident and the melanin particles blend with matrix material (42).

Histological studies have described melanosome disintegration in many cell types, but uniformly in phagosomes. Much attention has been given to keratinocytes and their lysosomally sequestered melanosome complexes (7–16), in which melanosomes lose their integrity and are converted into ill-defined melanosomal dust (14, 15). Taking into account the latest models of hierarchy in melanin supramolecular structure, melanin dust might represent one of the self-assembling units of eumelanin (17). It is important to add that the products of melanosome disintegration remain in the membrane-limited lysosomal compartment (and are not released in the cytoplasm) until their loss by epidermal desquamation (14, 15).

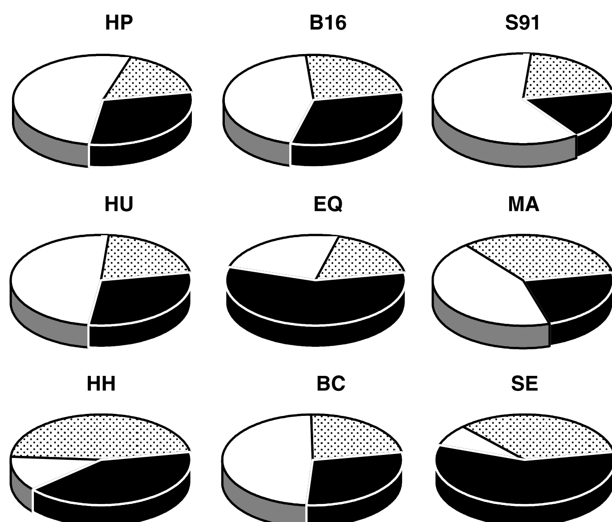


Fig. 1. Chemical composition of nine kinds of isolated melanosomes. Black segments, melanin; white segments, protein; dotted segments, the other substances (carbohydrates, lipids, inorganic substances); HP, Harding Passey mouse melanoma; B16, B16 mouse melanoma; S91, Cloudman S91 mouse melanoma; HU, human melanoma; EQ, horse melanoma; MA, Bomirski Syrian hamster melanoma; HH, human black hair; BC, bovine choroids; SE, Sepia ink; based on our results in (4, 5).

Melanosome disintegration has been noted also in heterophagosomes of professional phagocytes – macrophages (7, 18–20, 30), endothelial cells of skin vessels (21) and mast cells (22). The loss of melanosomal integrity has been observed also in autophagosomes of pigment producing cells – in melanocytes of hyperpigmented skin (23, 24) and hair bulbs (25), in the cells of junctional naevi (24), in morbus Bowen (26) and frequently in melanoma cells (16, 27, 28, 30, 31) (Fig. 2), in which the occurrence of aberrant melanosomes, often resembling partly dissociated melanosomes, is typical (29, 32, 33). Morphologically observed melanosome disintegration does not allow for reliable conclusions about the degradation of melanin as a chemical individuum (15).

Turnover of melanosomes in eye pigment tissues, i.e. in the retinal pigment epithelium (RPE), choroid and iris, has been studied thoroughly by Schraermeyer and his group (34–37). As for the melanin granule degradation, a step in melanosome turnover, they critically summarized the frequently observed association of melanosomes and lysosomes which according to some authors (38, 39) might suggest that melanosomes might be degraded, but they correctly concluded that it was questionable whether such an association indicates melanin degradation (34). Under normal conditions melanin(osome) laden macrophages can be found among choroid melanocytes exhibiting some signs of melanin disintegration (34).

In partly depigmented areas of the choroid in the Smyth chicken, a model for vitiligo, melanosomes displayed variant appearance with irregular shape, electron-lucent halos, pigmented vesicles, which all seemed to indicate melanosome/melanin degradation (40). Moreover, the choroids contained macrophages removing melanin (40). The hypopigmentation of choroid and RPE in the pallid mouse eye was explained by the digestion of immature melanosomes in secondary lysosomes (41).

Biochemical In vitro Studies

Attempts to induce melanosome disintegration or even complete degradation by biochemical means in vitro have failed. Japanese scientists, after treating radioactively labelled melanosomes with lysosomal hydrolases, noticed the quick degradation of lipids, the slow degradation of proteins but the stability of the melanin moiety of melanosomes (12, 43–45). The melanin framework of melanosomes resisted even harsh acid hydrolysis treatment with 6 mol HCl/l at 120°C for 72 h (46, 47) and melanosomes retained their size, shape and ultrastructural features (46), and even their appearance in scanning electron microscopy (47). The release of radioactivity from C^{14}-DOPA-labelled melanosomes was found to be negligible (44, 45, 48). Decarboxylation of melanin monomers accompanying acid hydrolysis (49, 69) had no impact on the melanin moiety architecture (46, 47).

The treatment of melanosomes with alkaline pH is associated with a release of melanin fragments (50); it induces formation of a soluble melanoprotein of fine granular structure (51) and is exploited in practice to distinguish between eumelanin and phaeomelanin in histological sections (33, 52). Nevertheless, such extreme pH in living cells would be incompatible with their survival.

All the above mentioned biochemical data suggest that hydrolytic mechanisms can hardly participate in the breakdown of the melanosomal melanin moiety.

Histochemical Studies

Histochemical papers demonstrated repeatedly an association of melanosomes with lysosomal enzymes, which might explain the digestion of non-melanin melanosome constituents. No lysosomal enzyme capable of melanin degradation has been found (8). The association of acid hydrolases with melanosomes (4, 53–55) can be explained not only by merging of melanosomes with lysosomes (7–16) but also simply by ranking melanosomes among lysosome-related organelles (56). Many reports on the presence of acid phosphatase in melanosome complexes (9, 57–59) and on its assumed role in melanosome degradation (57, 58) have appeared. The reaction specificity of acid phosphatase [EC 3.1.3.2] consists in disconnecting ester bonds of phosphoric acid. As there has been no report on the participation of phosphoric acid in maintaining the melanosome architecture or melanin supramolecular arrangement, its real role in melanosome/melanin disintegration and degradation is zero.

In vivo Studies

Schraermeyer and Dohms (36) investigated by electron microscopy whether melanosomes isolated from the choroid and RPE of cattle eyes could be degraded in phagolysosomes of cultured murine macrophages. After 3 d, a loss of melanosome homogenous electron density and an appearance of internal membranous structure consisting of concentric

lamellae was apparent suggesting melanin degradation. The observed lamellae were described as remnants of the original melanin polymer in the melanosome or may result from self-assembly of degradation products (36). Degradation of some melanosomes into smaller fragments without any internal structure was also observed.

Injection of heavily melanized dog hair melanosomes into peritoneal cavities of DBA2 and C57BL6J mice induced a granulomatous reaction with the occurrence of foreign body multinuclear giant cells but there was no evidence of any melanosomal disintegration. Melanosomes behaved like inert foreign bodies (60). Bomirski hamster melanoma melanosomes inoculated into Syrian hamster foot pads had granular incompact appearance but the look of the original isolated melanosomes used in the experiment was similar. In addition, there was no immunological cellular reaction of the host (60).

Moths fed dark hair did not digest melanins and excreted them in their faeces (61).

Melanin Structure Suggests a Possible Role of Redox Reactions in Pigment Degradation

The fundamental molecular unit of eumelanin (a detailed structure of phaeomelanin has remained obscure) is taken to be a small planar oligomer built from several 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid units which further assembles into structure of higher order – parallel layers stabilized by π-stacking and by side on interactions (62, 63). Hence, considering the melanin structure, redox mechanisms analogous with the biotransformation of polycyclic hydrocarbons (64, 65) seem to be more probably involved in pigment degradation than the hydrolytic reactions (60). Such an opinion is further supported by many reports demonstrating the redox breakdown of melanin in vitro:

1 In histology melanin is removed by pigment bleaching with oxidants such as permanganate (66) or hydrogen peroxide (67). In supramolecular terms melanin bleaching begins with an oxidative conversion of the multilayered stacked sheet to largely destacked mildly bleached thin melanin sheet as documented by atomic force microscopic measurements (68).
2 Chemical degradation of melanin pigments exploiting oxidation with permanganate or breakdown with HI has become a fundamental method for the quantitative estimation of eumelanin and phaeomelanin, respectively (69, 70).
3 Oxidative degradation of melanin after the addition of (71, 72) or boiling with H_2O_2 (73, 74) has been particularly thoroughly studied. Degradation can be quantitatively measured as a decrease of the absorbance (73,75,76); in the dark melanin is either resistant to the oxidation by H_2O_2 (76) or the decomposition is not associated with fluorescence (77).
4 Partial decomposition of melanin by boiling with hydrogen peroxide is accompanied by development of strong fluorescence which can be exploited in melanin quantification (73, 74). The nature of the melanin fluorogen(s) formed has remained unknown. It has been suggested that fluorescence indicates structural defects in melanin polymer (73, 78), for more details see (77); the bond between quinoid carbonyls, namely C5–C6 of the indole-5,6-quinone was calculated to be particularly weak and hence prone to nucleophil attack and to free radical attack (75).
5 Oxidative melanin degradation associated with fluorescence (71, 72, 79) – see Fig. 3, can be induced as a result of photoinduced hydrogen peroxide production (77). Reaction leading to the H_2O_2 photoproduction (80, 81) are summarized in Table 1. Photochemically induced free radicals can be involved in melanin degradation (82–84).

To summarize the in vitro observations we dare say that melanin can be oxidatively degraded. Bleaching and fluorescence are accompanying phenomena signalling the process of degradation. Can oxidative breakdown of melanin (and melanin moiety of melanosomes) so often observed in vitro occur also under in vivo conditions? The synthesis of hydrogen peroxide in melanosomes was demonstrated (85)

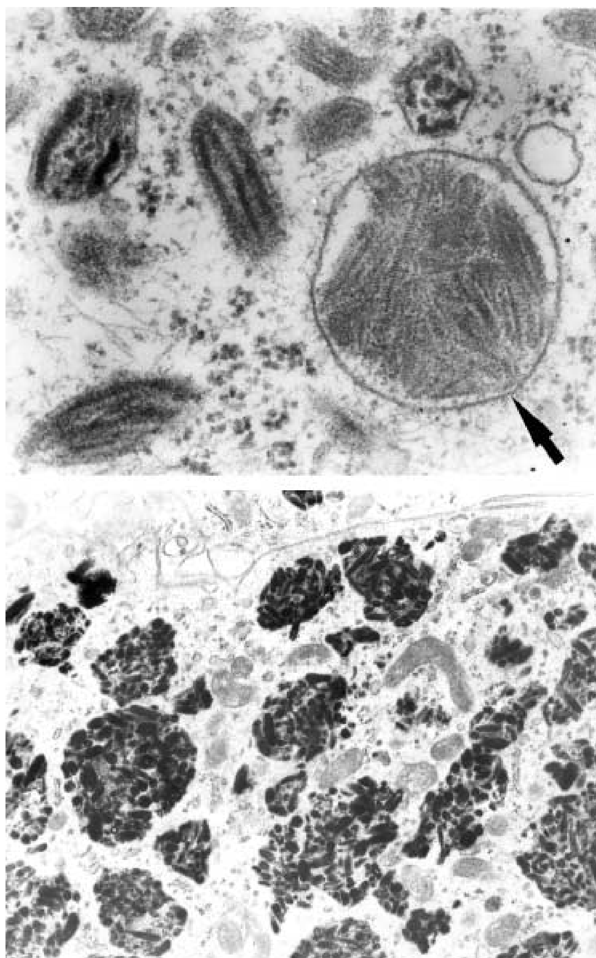


Fig. 2. Top – melanosomes and a large melanosome complex (arrow) in B16 melanoma. The complex can be not only place of melanosome disintegration/degradation but also source of material for melanosome synthesis as suggested in (35, 37). [Magn. 55 000:1]. Bottom – Autophagosomes containing melanosomes in B16 mouse melanoma. [Magn. 8700:1].

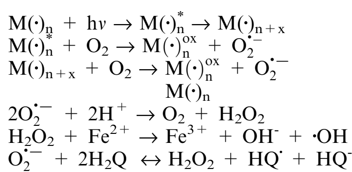
Fig. 3. Photoinduced melanin fluorescence in human foetal retinal pigment epithelium. Upper row, fluorescence microscopy; lower row, normal microscopy; Left column, unirradiated tissue; right column, tissue after 10-min irradiation with UVA and near UVA light.

and mechanisms of its possible photoproduction described (77, 80–82). Living cells are protected by antioxidant defences which prevent oxidative stress in living cells. Nevertheless, the irradiation of eye and skin melanosomes can be so intensive that the antioxidant defence may be overcome.

Can Enzymes involved in the Biotransformation of Polycyclic Aromatic Hydrocarbons Participate in Melanin Degradation?

Under the in vivo situation, polycyclic aromatic hydrocarbons are usually biotransformed enzymatically (64, 65, 86, 87). Two groups of enzymes are specialized in this respect: monooxygenases and dioxygenases. Cytochrome P450 (CYP) system (EC1.14.14.1), a monooxygenase, is a biotransforming system localized in the endoplasmic reticulum and in mitochondria (65, 86–89). As under physiological conditions melanosomes can hardly reach these compartments, the participation of CYP in the biodegradation of the melanin moiety of melanosomes, seems to be improbable. Moreover, CYP activity was shown to be easily destroyed by quinones (89), and quinones are produced during melanogenesis (90–92) and quinoid groups are present in melanin biopolymers (1, 70, 91). Proteomic analysis of phagosomal proteins has recently revealed the presence of cytochrome P450 probably because of the endoplasmic reticulum recruitment to phagosomes (93), but whether the endoplasmic reticulum enzyme activities survive, has remained unknown.

Table 1. Reactions leading to H_2O_2 and reactive oxygen species photoproduction



$M(\cdot)_n + h\nu \rightarrow M(\cdot)_n^{*} \rightarrow M(\cdot)_{n+x}$
$M(\cdot)_n^{*} + O_2 \rightarrow M(\cdot)_n^{ox} + O_2^{\bullet -}$
$M(\cdot)_{n+x} + O_2 \rightarrow M(\cdot)_n^{ox} + O_2^{\bullet -}$
$M(\cdot)_n$
$2O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2$
$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {\cdot}OH$
$O_2^{\bullet -} + 2H_2Q \leftrightarrow H_2O_2 + HQ^{\cdot} + HQ^-$

For the explanation, see (77, 80)

Dioxygenases take part in the polycyclic hydrocarbons' degradation primarily through the introduction of both atoms of molecular oxygen (87, 88, 94), but they are localized only in the cytoplasmic compartment of the cell.

We all remember that disintegration of melanosomes is situated in phagosomes. Phagosomal (and plasma) membranes contain NADPH oxidase, a multicomponent enzyme,

which in the case of the phagosome, produces superoxide anion radical and hydrogen peroxide to its interior (65, 95). Melanin structures are sensitive to H_2O_2 and reactive oxygen species attacks (67, 73–75, 77, 80–84). NADPH oxidase meets the requirement of phagosomal localization and reaction specificity, and may be a hot candidate for a key role in the degradation of melanosomal melanin moiety. However, its real role remains to be proven.

Unlike aromatic polycyclic hydrocarbons, a convincing direct demonstration of melanin degradation in living animal cells has been lacking. There are only reports demonstrating melanin degradation by *Aspergillus fumigatus* (96) and a very slow degradation of fungal melanins (uniformly ^{14}C-labelled fungal melanins incubated in various soils were losing 5–10% ^{14}C evolved as ^{14}C -carbon dioxide for 12 weeks) (97). These reports together with morphologically detected disappearance of both melanin pigment in microscopy (77) (Fig. 3) and electron dense material in electron microscopy (34, 36) suggest that complete melanosome biodegradation might be possible, particularly via oxidative reactions, in spite of the fact that melanin is a highly resistant structure (98, 99).

Some Questions for the Future

1. Is the biodegradation of melanin in vivo a common or a rare phenomenon?
2. What is the nature and fate of melanin degradatory products in vivo? Can we presume that degradatory fluorescing products, especially in the case of extracutaneous melanin, may accumulate as simultaneously deposited autofluorescent lipofuscin (77)?
3. Phaeomelanin solubility, reactivity and free radical contents are higher compared with eumelanin (100) and phaeomelanosomes appear to be more primitive melanosomes lacking some components (4). Hence, would the biodegradability of phaeomelanin be easier compared with eumelanin?
4. A set of acid hydrolases is associated with melanosomes from early stages of their maturation. What triggers the hydrolysis of non-melanin moieties of melanosomes?
5. Is there any relationship between the degree of melanosome maturation and the biodegradability? The presence of ongoing melanogenesis results in an increase of a subset of lysosomal hydrolases (4, 54, 59) and the more melanized melanosomes appear to have a progressively lower pH (101).

Acknowledgements – This work was supported by the IGA MZ grant no. 6804-3 (Czech Republic) to JB and by the research project VZ000011113 (Czech Rep.) to ME.

REFERENCES

1. Prota G. Melanins, melanogenesis and melanocytes: looking at their functional significance from chemist's point of view. Pigment Cell Res 2000;13:283–293
2. Kushimoto T, Basrur V, Valencia J, Matsunaga J, Vieira WD, Ferrans VJ, Muller J, Appella E, Hearing VJ. A model for melanosome biogenesis based on the purification and analysis of early melanosome. Proc Natl Acad Sci USA 2001;98:10 698–10 703
3. Raposo G, Tenza D, Murphy DM, Berson JF, Marks MS. Distinct protein sorting and localization to premelanosomes, melanosomes and lysosomes in pigmented melanocytic cells. J Cell Biol 2001;152:809–823
4. Orlow SJ. Biogenesis of melanosomes. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne JP. The Pigmentary System. Oxford: Oxford University Press; 1998. pp. 97–106
5. Duchon J, Borovanský J, Hach P. Chemical composition of ten kinds of various melanosomes. In: McGovern VJ, Russell P. Mechanisms in Pigmentation. Munich, Paris London, New York, Sydney: S Karger Basel; 1973. pp. 165–170
6. Hach P, Duchon J, Borovanský J. Ultrastructural and biochemical characterization of isolated melanosomes. Folia Morph (Prague) 1977;21:407–410
7. Mishima Y. Macromolecular changes in pigmentary disorders. Arch Dermatol 1965;91:519–557
8. Zelickson AS, Windhorst DB, White JG, Good RA. The Chediak–Higashi syndrome. Formation of giant melanosomes and the basis of hypopigmentation. J Invest Dermatol 1967;49:575–581
9. Hori Y, Toda K, Pathak MA, Clark WH Jr, Fitzpatrick TB. A fine structure study of the human epidermal melanosome complex and its acid phosphatase activity. J Ultrastruct Res 1968;25:109–120
10. Olson RL, Nordquist J, Everett MA. The role of epidermal lysosomes in melanin physiology. Br J Dermatol 1970;83:189–199
11. Jimbow K, Sato S, Kukita A. Lysosomal degradation of melanosomes. Skin Res 1971;13:19–34 (In Japanese)
12. Ohtaki N, Seiji M. Degradation of melanosomes by lysosomes. J Invest Dermatol 1971;57:1–5
13. Fitzpatrick TB, Quevedo WC Jr. Biological processes underlying melanin pigmentation and melanin disorders. Mod Trends Dermatol 1971;4:122–149
14. Wolff K. Melanocyte–keratinocyte interactions in vivo: the fate of melanosomes. Yale J Biol Med 1972;46:384–396
15. Wolff K, Honigsmann H. Are melanosome complexes lysosomes? J Invest Dermatol 1972;59:170–176
16. Bleehen SS. Ultrastructural studies on tumours and cell cultures of the Harding–Passey mouse melanoma. Br J Dermatol 1974;90:637–648
17. Clancy CMR, Nofsinger JB, Hanks RK, Simon JD. A hierarchical self-assembly of eumelanin. J Phys Chem B 2000;104:7871–7873
18. Ohtaki N. Melanosome and lysosome. I. Lysosomal activity in relation to growth of melanoma. Bull Tokyo Med Dent Univ 1970;17:89–102
19. Sato S, Nishijima A, Hiraga K. Lymphatic transport and phagocytosis of melanosomes in blue nevus. Arch Derm Forsch 1975;252:239–244
20. Mishima Y. Cellular and subcellular differentiation of melanin phagocytosis and synthesis by lysosomal and melanosomal activity. J Invest Dermatol 1966;46:70–75
21. Sato S, Kukita A. Electron microscopic study of melanin-phagocytosis by cutaneous vessels in cellular blue nevus. J Invest Dermatol 1969;52:528–532
22. Sato S, Kukita A, Sato S. Phagocytosis and degradation of melanosomes by the mast cells. J Invest Dermatol 1969;53:183–186
23. Jimbow K, Quevedo WC Jr, Fitzpatrick TB, Szabo G. Some aspects of melanin biology: 1950–1975. J Invest Dermatol 1976;67:72–89
24. Wolff K, Schreiner E. Melanosomal acid phosphatase. Arch Derm Forsch 1971;241:255–272
25. Jimbow K, Szabo G, Fitzpatrick TB. Ultrastructural investigation of autophagocytosis of melanosomes and programmed death of melanocytes in white Leghorn feathers: a study of morphogenetic events leading to hypomelanosis. Dev Biol 1974;36:8–23
26. Kawamura T, Ikeda S, Mori S, Obata H. Electron microscopic findings compatible with those of the lysosome (autophagic vacuole) revealed in the melanocytes in cases of conspicuous pigment blockade. Jap J Dermatol Series B 1966;76:405–408
27. Hirone T, Nagai T, Matsubara T, Fukushiro R. Human malignant melanomas of the skin and their pre-existing conditions. In: Kawamura, T, Fitzpatrick, TB, Seiji, M. Biology of Normal and Abnormal Melanocytes. Baltimore, London, Tokyo: University Park Press; 1971. pp. 329–348
28. Césarini JP. Recent advances in the ultrastructure of malignant melanoma. Rev Europ Études Clin Biol 1971;16:316–322
29. Hunter JAA, Zaynoun S, Paterson WD, Bleehen SS, MacKie R, Cochran AJ. Cellular fine structure in the invasive nodules of different histogenetic types of malignant melanoma. Br J Dermatol 1978;98:255–272

30. Mishima Y. Lysosomes in melanin phagocytosis and synthesis. Nature 1967;216:67
31. Seiji M, Ohtaki N. Lysosomes in mouse melanoma. J Invest Dermatol 1971;56:436–440
32. Borovanský J, Miřejovský P, Riley PA. Possible relationship between abnormal melanosome structure and cytotoxic phenomena in malignant melanoma. Neoplasma 1991;38:393–400
33. Nagai N, Lee YJ, Nagaoka N, Gunduz M, Nakano K, Nojima T, Tsujigiwa H, Gunduz E, Siar CH, Nagatsuka H. Elemental sulphur and alkali elutable melanin detected in oral melanosis and malignant melanoma by energy-filtering transmission electron microscopy. J Oral Pathol Med 2002;31:481–487
34. Schraermeyer U. Does melanin turnover occur in the eyes of vertebrates. Pigment Cell Res 1993;6:193–204
35. Schraermeyer U, Stieve H. A newly discovered pathway of melanin formation in cultured retinal pigment epithelium. Cell Tissue Res 1994;276:273–279
36. Schraermeyer U, Dohms M. Detection of a fine lamellar gridwork after degradation of ocular melanin granules by cultured peritoneal macrophages. Pigment Cell Res 1996;9:248–254
37. Schraermeyer U. Evidence for melanogenesis in the retinal pigment epithelium of adult cattle and golden hamster. Comp Biochem Physiol 1992;103:435–442
38. Feeney L. Lipofuscin and melanin of human retinal pigment epithelium. Fluorescence, enzyme cytochemical and ultrastructural studies. Invest Ophthalmol Visual Sci 1978;17:583–600
39. Novikoff AB, Neuenberger PM, Novikoff PM, Quintana N. Retinal pigment epithelium. Interrelations of endoplasmic reticulum and melanolysosomes in the black mouse and its beige mutant. Laboratory Invest 1979;40:155–165
40. Boissy RE, Gecks S, Smyth JR, Nordlund JJ. Ocular pathology in the minimally depigmented subline of the vitiliginous Smyth chicken. Pigment Cell Res 1988;1:303–314
41. Ito M, Hashimoto K, Organisciak DT. Ultrastructural, histochemical and biochemical studies of the melanin metabolism in pallid mouse eye. Curr Eye Res 1982/1983;2:13–28
42. Herrman KG, Steinberg RH. Melanosome metabolism in the retinal pigment epithelium of the opossum. Cell Tissue Res 1982;227:485–507
43. Ohtaki N. Melanosome and lysosome. II. Digestion of melanosome with mouse liver lysosome. Bull Tokyo Med Dent Univ 1970;17: 170–186
44. Saito N, Seiji M. Epidermal lysosome and the degradation of melanosomes. Acta Dermatovener (Stockholm) 1973;Suppl. 73:69–74
45. Saito N, Seiji M. Degradation of melanosomes in vitro and in vivo. Pigment Cell 1976;3:384–392
46. Borovanský J, Hach P, Duchon J. Melanosome: an unusually resistant subcellular particle. Cell Biol Int Rep 1977;1:549–554
47. Broekhuyse RM, Kuhlmann ED, Winkens HJ. Experimental autoimmune anterior uveitis. III. Induction by immunization with purified uveal and skin melanins. Exp Eye Res 1993;56: 575–583
48. Borovanský J, Pavel S, Duchon J, Vulterin K. Incorporation of L-3,4-dihydroxy-(2–^{14}C)-phenylalanine into hamster melanoma melanosomes. FEBS Lett 1979;104:291–293
49. Zeise L, Murr BL, Chedekel MR. Melanin standard method: particle description. Pigment Cell Res 1992;5:132–142
50. Borovanský J, Duchon J, Procházková B, Hach P. An attempt to disintegrate melanosomes into protein subunits. Čas Lék Čes 1972;111:218–220 (In Czech)
51. Borovanský J, Duchon J, Hach P. Soluble melanoprotein of tumor origin. Neoplasma 1975;22:525–530
52. Inazu M, Mishima Y. Detection of eumelanogenic and pheomelanogenic melanosomes in the same normal human melanocyte. J Invest Dermatol 1993;100:172S–175S
53. Orlow SJ. Melanosomes are specialized members of the lysosomal lineage of organelles. J Invest Dermatol 1995;105:3–7
54. Diment S, Eidelman M, Rodriguez GM, Orlow SJ. Lysosomal hydrolases are present in melanosomes and are elevated in melanizing cells. J Biol Chem 1995;270:4213–4215
55. Smit NPM, van Roermund CWT, Aerts HMFG, Heikoop JV, van den Bergh M, Pavel S, Wanders RJA. Subcellular fractionation of cultured human melanocytes: new insights into the relationship of melanosomes with lysosomes and peroxisomes. Biochim Biophys Acta 1993;1181:1–6
56. Dell'Angelica EC, Mullins C, Caplan S, Bonifacino JS. Lysosome-related organelles. FASEB J 2000;14:1265–1278
57. Jimbow K, Sugiyama S. Melanosomal translocation and transfer. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne JP. The Pigmentary System. Oxford: Oxford University Press; 1998. pp. 107–114.
58. Nakagawa H, Rhodes AR, Fitzpatrick TB, Hori Y. Acid phosphatase in melanosome formation: a cytochemical study in normal human melanocytes. J Invest Dermatol 1984;83:140–144
59. Kikuchi A. Acid phosphatase activity in melanosomes of melanocytes. Bull Tokyo Med Dent Univ 1968;15:279–294
60. Borovanský J, Hach P, Smetana K Jr, Elleder M, Matous-Malbohan I. Attempts to induce melanosome degradation *in vivo*. Folia Biologica (Prague) 1998;45:47–52
61. Zbytniewski Z, Drewa G. Proteolytic activity of the homogenates of transplantable melanotic and amelanotic melanoma in golden hamster (Mesocricetus auratus, Waterhouse). Polish Med J 1972;11:397–404
62. Zajac GW, Gallas JM, Cheng J, Eisner M, Moss SC, Alvarado-Swaisgood AE. The fundamental unit of synthetic melanin: a verification by tunneling microscopy of X-ray scattering results. Biochim Biophys Acta 1994;1199:271–278
63. Clancy CMR, Simon JD. Ultrastructural organization of eumelanin from Sepia officinalis measured by atomic force microscopy. Biochemistry 2001;40:13 353–13 360
64. De Pierre JW, Ernster L. The metabolism of polycyclic hydrocarbons and its relationship to cancer. Biochim Biophys Acta 1978;473:149–186
65. Murray KR. Red and white blood cells. Metabolism of xenobiotics. In: Harper's Biochemistry, 25th edn. New York: Appleton & Lange; 2000. pp. 763–786
66. Kligora CJ, Fair KP, Clem MS, Patterson JW. A comparison of melanin bleaching and Azure blue counterstaining in the immunohistochemical diagnosis of malignant melanoma. Modern Pathol 1999;12:1143–1147
67. Lyon H. Theory and Strategy in Histochemistry. Heidelberg: Springer; 1991. pp. 237–250
68. Gallas JM, Zajac W, Sarna T, Stotter PL. Structural differences in unbleached and mildly bleached synthetic tyrosine-derived melanin identified by scanning probe microscopies. Pigment Cell Res 2000;13:99–108
69. Ito S, Fujita K. Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography. Anal Biochem 1985;144:527–536
70. Ito S. Advances in chemical analysis of melanin. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne JP. The Pigmentary System Oxford: Oxford University Press; 1998, pp. 439–450
71. Kayatz P, Thumann G, Luther TT, Jordan JF, Bartz-Schmidt KU, Esser PJ, Schraermeyer U. Oxidation causes melanin fluorescence. Invest Ophthalmol Vis Sci 2001;42:241–246
72. Mosca L, De Marco C, Fontana M, Rosei AM. Fluorescence properties of melanins from opioid peptides. Arch Biochem Biophys 1999;371:63–69
73. Korzhova LP, Frolova EV, Romakov YA. Spectrofluorimetric procedure for registration of products developed after oxidative destruction of eumelanins. Vopr Med Khim 1989;35:139–143 (In Russian)
74. Rosenthal MH, Kreider JW, Shiman R. Quantitative assay of melanin in melanoma cells in culture and tumors. Anal Biochem 1973;56:91–99
75. Slawinska D, Slawinski J. Electronically excited molecules in the function and degradation of melanins. Physiol Chem Phys 1982;14:363–372
76. Korytowski W, Sarna T. Bleaching of melanin pigments. J Biol Chem 1990;265:12 410–12 416
77. Elleder M, Borovanský J. Autofluorescence of melanins induced by ultraviolet radiation and near ultraviolet light. A histochemical and biochemical study. Histochem J 2001;33:273–281
78. Gallas JM, Eisner M. Fluorescence of melanin-dependence upon excitation wavelength and concentration. Photochem Photobiol 1987;45:595–600
79. Korzhova LP, Frolova EV, Romakov YA, Kuznetsova NA. The photoinduced destruction of dopa melanin. Biokhimija 1989;54:992–998 (In Russian)
80. Nofsinger JB, Liu Y, Simon JD. Aggregation of melanin mitigates photogeneration of reactive oxygen species. Free Rad Biol Med 2002;32:720–730

81. Korytowski W, Hintz P, Sealy RC, Kalyanaraman B. Mechanism of dismutation of superoxide produced during autoxidation of melanin pigment. Biochem Biophys Res Commun 1985;131:659–665
82. Felix CC, Hyde JS, Sarna T, Sealy RC. Melanin photoreactions in aerated media. Electron spin resonance evidence for production of superoxide and hydrogen peroxide. Biochem Biophys Res Commun 1978;84:335–341
83. Felix CC, Hyde JS, Sealy RC. Photoreactions of melanin: a new transient species and evidence for triplet state involvement. Biochem Biophys Res Commun 1979;88:456–461
84. Slawinska D, Slawinski J. Ultraweak proton emission in model reactions of the in vitro formation of eumelanins and pheomelanins. Pigment Cell Res 1987;1:171–175
85. Glickman RD, Sowell R, Lam KW. Kinetic properties of light-dependent ascorbic acid oxidation by melanin. Free Rad Biol Med 1993;15:453–457
86. Guengerich FP. Human cytochrome P450 enzymes. In: Ortiz de Montellano PR. Cytochrome P450: Structure, Mechanism and Biochemistry. New York: Plenum Press; 1995. pp. 473–535.
87. Stiborová M, Hudecek J, Páca J. Enzyme systems biotransforming phenol compounds. Bull Cs Spol Biochem Mol Biol 2000;28:57–73 (In Czech)
88. Mitchell GA, Grompe M, Lambert M, Tanguay RM. Hypertyrosinemia. In: Scriver C, Beaudet AL, Sly WS, Valle D. The Metabolic & Molecular Basis of Inherited Disease, Vol. III. New York & St Louis: McGraw-Hill Med. Publ. Div; 2001. pp. 1777–1805
89. Soucek P. Cytochrome P450 destruction by quinones: comparison of effects in rat and human liver microsomes. Chemico-Biol Interactions 1999;121:223–236
90. Pavel S, Smit NPM. Detoxification processes in pigment-producing cells. In: Hori Y, Hearing VJ, Nakayama J. Melanogenesis and Malignant Melanoma: Biochemistry, Cell Biology, Molecular Biology, Pathophysiology, Diagnosis and Treatment. Amsterdam, Lausanne: Elsevier Science BV; 1996. pp. 161–168
91. Riley PA. Melanin. Int J Biochem Cell Biol 1997;29:1235–1239
92. Lambert C, Chacon JM, Chedekel MR, Land EJ, Riley PA, Thompson A, Truscott TG. A pulse radiolysis investigation on indolic melanin precursors-evidence for indolquinones and subsequent intermediates. Biochim Biophys Acta 1989;993:12–20
93. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M. The phagosome proteome: insight into phagosome functions. J Cell Biol 2001;152:165–180
94. Khan AA, Wang RF, Cao WW, Doerge DR, Wennerstrom D, Cerniglia CE. Molecular cloning, nucleotide sequence, and expression of genes encoding a polycyclic aromatic ring dioxygenase from *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 2001;67:3577–3585
95. Dinauer MC, Nauseef WN, Newburger PE. Inherited disorders of phagocyte killing. In: Scriver C, Beaudet AL, Sly WS, Valle D. The Metabolic & Molecular Basis of Inherited Disease, Vol. II. New York & St Louis: McGraw-Hill Med Publ Div; 2001, pp. 4857–4887.
96. Luther JP, Lipke H. Degradation of melanin by *Aspergillus fumigatus*. Appl Environ Microbiol 1980;40:145–155
97. Martin JP, Haider K. Biodegradation of 14C-labeled model and cornstalk lignins, phenols, model phenolase humic polymers and fungal melanins as influenced by a readily available carbon source and soil. Appl Environ Microbiol 1979;38:283–289
98. Thathachari YT. Spatial structure of melanins. Pigment Cell 1976;3:64–68
99. Blois MS Jr. Physical studies of melanins. In: Kawamura T, Fitzpatrick TB, Seiji M. Biology of Normal and Abnormal Melanocytes. Baltimore, New York, Tokyo: University Park Press; 1971. pp. 125–136
100. Chedekel MR. Photochemistry and photobiology of epidermal melanins. Photochem Photobiol 1982;35:881–885
101. Bhatnagar V, Anjaiah S, Puri N, Darshanam BNA, Ramaiah A. pH of melanosomes of B16 murine melanoma is acidic: its physiological importance in the regulation of melanin synthesis. Arch Biochem Biophys 1993;307:18–192