Exp Dermatol 1999: 8: 153–164
Printed in Denmark · All rights reserved

Experimental Dermatology
ISSN 0906-6705

Controversies in Experimental Dermatology

Section Editor: Ralf Paus, Hamburg

What's the use of generating melanin?

Wood JM, Jimbow K, Boissy RE, Slominski A, Plonka PM, Slawinski J, Wortsman J, Tosk J. What's the use of generating melanin? Exp Dermatol 1999: 8: 153–164. © Munksgaard, 1999

J. M. Wood, K. Jimbow, R. E. Boissy, A. Slominski, P. M. Plonka, J. Slawinski, J. Wortsman and J. Tosk

The CONTROVERSIES featured in the August 1998 issue of Exp Dermatol have discussed the controls of melanogenesis (Schallreuter et al., Exp Dermatol 7: 143–150, 1998). Now, we explore the biological functions of the endproduct of melanogenesis, the various melanin biopolymers. As delineated in the subsequent contributions, melanins are functionally much more complex and fascinating than their evident colour-awarding and UV-light-filtering properties suggest, and dermatologists and pigment biologists alike have yet to discover and define the full range of biologically relevant melanin functions in health and disease.

Viewpoint 1

Melanins are found in highly oxidizing situations where significant concentrations of oxygen radicals are generated. Such reactive oxygen species (ROS) are produced by UV-light in the skin and eye, by sound and ultrasound in the inner ear, and in the highly oxidizing conditions of catecholaminergic neurons in the brain. The role of the melanins as oxygen radical trapping polymers, UV-light filters, polymeric buffers for transition metals, calcium and catecholamines will be considered in this viewpoint. For the Dermatologist it is of special interest to understand those factors that control melanogenesis in the skin and hair (3). In this context the pterins, alpha melanocyte stimulating hormone (α-MSH) and calcium appear to be central in the regulation of both melanin content and composition in melanocytes. There is accumulating evidence that the evolution of melanogenesis in human melanocytes is primarily antioxidant in character and is designed to protect these pigment cells from the cytotoxicity of ROS. However, the subtle link between calcium homeostasis and melanogenesis suggests that natural skin colours in the human population may have evolved secondarily as a consequence of salt balance/dehydration factors as primates adapted to extreme differences in local climates. It is tempting to speculate that skin colour emerged as a side effect through Darwinian natural selection principles in tropical versus temperate climates.

There is increasing evidence that melanogenesis represents a major antioxidant defence mechanism in melanocytes. Melanocytes in the human epidermis (*in vivo*) or in cell cultures (*in vitro*) have been shown to be much more sensitive to the cytotoxicity of ROS than keratinocytes (17, 27). This concept is especially well-documented by the cytotoxicity of hydrogen peroxide (H_2O_2) to melanocytes both (*in vivo*) and (*in vitro*) (6, 14, 15, 17). Consequently, melanocytes have evolved an effective strategy to deal with the removal of superoxide anion radicals (O_2^-) before these radical anions disproportionate to H_2O_2.

The preferential utilization of O_2^- compared to dioxygen (O_2) is quite a common event in the skin. The enzymes tyrosinase, proline hydroxylase and indoleamine 2,3 dioxygenase selectively utilize O_2^- as the preferred substrate over O_2 for the formation of dopaquinone, hydroxyproline residues in collagen, and kynurenic acid respectively (2, 20, 21,

23, 25). Therefore, the concept of the production of O_2^- trapping polymers applies not only to the formation of the melanins in melanocytes but also to the formation of collagen in fibroblasts.

Melanogenesis in melanocytes depends on the activity of tyrosinase, an enzyme which is activated by O_2^- through sequential single electron reductions of the two cupric ions (Cu^{II}) in its active site to two cuprous ions (Cu^{I}) yielding fully active enzyme (25). This enzyme directly produces dopaquinone as the product and not L-dopa, since the turnover of the latter intermediate is too rapid for its existence intracellularly. The free radical coupling reactions that follow dopaquinone formation provide a major sink for O_2^-, and therefore melanins are free radical-neutralizing polymers.

In the case of melanogenesis, the melanins are packaged into melanosomes, and these organelles are transferred to surrounding keratinocytes, followed by the programmed loss of these polymers into the stratum corneum during the keratinocyte life cycle. In addition to their property as a free radical trap, the melanins have relatively weak UV-absorption capability. Eumelanins in pigmented epidermis have an estimated sun protection factor value (SPF) of between 2–3 compared to depigmented epidermis (5). Meanwhile pheomelanins are extremely photo-active, generating high concentrations of ROS in the skin and offering even lower SPF values than eumelanins (7).

Even though the melanins are currently considered to be random polymeric structures, assembled through free radical coupling reactions, the abundance of phenolate, quinonoid, carboxylate and sulphur (S) donor groups allow stable complexation of transition metals such as iron and copper. Such uncomplexed transition metals together with UV-light generate hydroxyl radicals (OH°) from H_2O_2 in the skin by the Haber–Weiss reaction (13). OH° radicals are highly reactive with proteins, lipids and nucleic acids and pose a major cytotoxic threat to all cells in the skin (2, 13). Therefore, the saturation binding of transition metals to melanin affords yet another antioxidant defence utility for these polymers in the skin.

The preponderance of O-donor atoms in the melanins may provide stable co-ordination complexes with calcium, which requires 8 O-donor atoms for stable co-ordination per calcium ion. Undoubtedly calcium binding and exchange represents an important function for the melanins in the inner ear (8, 24). This property of the melanins to act as a buffer for binding calcium has not been fully explored in the skin where these ions play a key role in both cell differentiation and pigmentation (16, 19).

Melanins in the brain appear to have a different origin from those in the skin, ears and eyes. There is no evidence for the expression of tyrosinase in the brain and dopamine has been implicated as the substrate for neuromelanins. The so-called neuromelanins are primarily located in the substantia nigra, and these polymers are associated with catecholaminergic neuronal cells. Recent research on Parkinson's disease, where the neuromelanins are lost, suggests important functions for these polymers in trapping transition metals, catecholamine buffering and defence against damaging ROS (9).

Table 1 summarizes some selected useful properties for the melanins. Clearly, the melanins can be useful polymers, but if so, why do we have such variability in both their composition and content in human skin? Also, what are the advantages (if any) of

Table 1. Properties of melanins

EUMELANIN	Random polymeric structure primarily formed from the metabolism of L-tyrosine by a series of free radical coupling reactions (9) Relatively weak UV filter with SPF≅2 (5) Biosynthesis involves capture and neutralization of oxygen radicals (a potent anti-oxidant defence mechanism for melanocytes) (20–24) Complexes transition metal ions as well as calcium and, therefore, influences calcium homeostasis by acting as a calcium storage polymer (8) Synthesis controlled by α-MSH/MC-1 receptor function (21)
PHEOMELANIN	Random polymeric structure primarily formed from L-tyrosine and L-cysteine by Michael addition and free radical coupling reactions (9) Virtually no UV filter properties (7) Both generates and traps oxygen radicals upon exposure to UV light. Therefore its synthesis can be self generating (7) Complexes transition metals and calcium (7) Synthesis not controlled by α-MSH/MC-1 receptor function because MC-1 receptor is non-functional in pheomelanogenesis (21)
NEUROMELANIN	Polymeric structure primarily found in the substantia nigra and locus coeruleus in the brain and believed to be formed from the polymerization of dopamine together with L-cysteine (9) Complexes transition metal ions and traps oxygen radicals (9) Decreases with age (after 60) and is entirely lost in Parkinson's disease (9) May act as a catecholamine sink in maintaining neurotransmitter homeostasis in catecholaminergic neurons (9)

de novo melanogenesis catalysed by UVB light (290–320 nm), a process described by dermatologists as delayed tanning.

Recent results suggest that the composition of melanins are determined by the interaction of α-MSH with its MC-1 receptor on melanocytes. Polymorphism in the MC-1 receptor gene leads to receptor inactivation and promotes pheomelanogenesis (22). This important work is supported by inactivation of the MC-1 receptor by agouti protein which also yields a preference for pheomelanin synthesis (4). The huge variability in the content of melanins in human skin and hair poses a much more difficult question. However, lessons can be learned from studying patients with depigmentation disorders such as vitiligo and Hermansky–Pudlak Syndrome (HPS).

In each of these pathologic conditions there is an established breakdown in calcium uptake/efflux in epidermal cells (12, 14). The extracellular concentration of calcium in the culture media of melanocytes and melanoma cells is well known to control normal pigmentation. Furthermore, calcium controls melanosome transfer from melanocytes to keratinocytes (11). These results suggest a connection between, e.g. the salt balance of fast exchange ions, e.g. (calcium) and pigmentation. Salt balance would be critical for the survival of primates in the intense heat of the tropical environment. Therefore, the evolution of primates in such an environment would require the natural selection of individuals with very efficient fast exchange ion transport and efflux, and as a consequence increased melanogenesis. Meanwhile in the more temperate climates ion imbalances caused by heat, i.e. sunstroke, would be less problematic and more polymorphism in genes related to melanogenesis could be tolerated yielding the observed phenotypic variations in skin and hair colours worldwide.

Is it possible that melanogenesis is really an evolutionary side effect related to the primary process of fast exchange ion homeostasis? Albeit it is ironical that such a proposed evolutionary accident still provides melanocytes with a major antioxidant defence mechanism against ROS in the skin and so melanins can certainly be useful. To answer the second question on the reason for UVB catalysed *de novo* melanogenesis it is necessary to examine the biochemical process of delayed tanning.

Fig. 1 presents our recent understanding of the control of *de novo* melanogenesis in human skin. Firstly, melanocytes express all of the enzymes for the rapid synthesis and recycling of the essential cofactor 6(R)-L-erythro 5, 6, 7, 8 tetrahydrobiopterin ($6BH_4$). This cofactor controls the supply of L-tyrosine from L-phenylaline and allosterically inhibits tyrosinase. However, $6BH_4$ is photo-oxidized

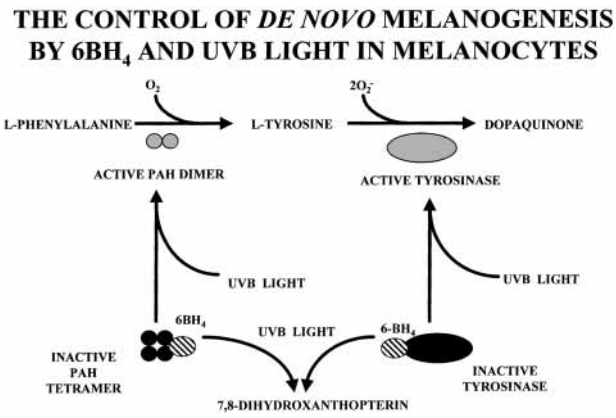

Figure 1. L-phenylalanine is actively transported into human melanocytes where it is rapidly oxidized to L-tyrosine by phenylalanine hydroxylase (PAH). A large supply of L-tyrosine is necessary to sustain melanogenesis via the rate limiting and key enzyme tyrosinase, $6BH_4$ inhibits PAH by forming inactive tetromers from active dimers (1) and also inhibits tyrosinase by allosteric control (26). When $6BH_4$ is photo-oxidized by UVB light it is converted to 7,8 dihydroxanthopterin leading to the activation of both PAH and tyrosinase and pigmentation occurs. UVA light does not promote the photo-oxidation of $6BH_4$. Therefore, $6BH_4$ functions as a molecular light switch for *de novo* melanogenesis turned on by UVB light.

specifically by UVB light to activate both phenylalanine hydroxylase and tyrosinase, yielding both an increase in the supply of L-tyrosine while at the same time stimulating tyrosinase activity (18, 26). Therefore the pterin ($6BH_4$) can be regarded as a photo-switch turning on the delayed tanning process.

The question remains: "How can this process be useful to the protection of human skin from ROS?" The answer to this question may reside in not only the excess synthesis of melanins but also in the specific structure of the photo-oxidation product of $6BH_4$, which has been identified as 7,8 dihydroxanthopterin (18). These dihydropterins are known to be lipid soluble, and are effective terminators of lipid peroxidation cascades. Therefore, this photo-switch may protect cell membrane integrity in melanocytes during *de novo* melanogenesis (10). Consequently, even *de novo* melanogenesis has antioxidant components designed to protect the viability of the small numbers of human melanocytes found in the human epidermis.

John M. Wood
Clinical and Experimental Dermatology
Dept of Biomedical Sciences
University of Bradford
Bradford DB7 IDP, UK

References

1. Davis M D et al. Arch Biochem Biophys 1996: 325(2): 235–241.

2. Hayaishi O. In: Hayaishi O et al. eds. The Biological Role of Reactive Oxygen Species in Skin. Amsterdam: Elsevier, 1987: 3–8.
3. Illis I. Dermatologic Clinics 1988: 6: 369–375.
4. Jackson I J. Nature 1993: 362: 587–588.
5. Kaidberg K H, Kligman A M. Arch Dermatol 1978: 114: 46–48.
6. Maresca V et al. J Invest Dermatol 1997: 109: 310–313.
7. Menon I A et al. In: Bagnara J T et al., eds. Pigment Cell Biological, Molecular and Clinical Aspects of Pigmentation. Tokyo: University of Tokyo Press, 1985: 77–85.
8. Meyer-zum Gottesberge A M. Pigment Cell Res 1988: 1: 238–249.
9. Prota G. Melanins and Melanogenesis, Academic Press 1992: 119–133.
10. Reibnegger G et al. In: Pflieder N, Rokos H, eds. Chemistry and Biology of Pteridines and Folates. Oxford: Blackwell Scientific 1997: 783–790.
11. Sawyer T K et al. Amer Zool, 1983: 23: 529–540.
12. Schallreuter K U, Pittelkow M R. Arch Derm Res 1988: 280: 137–139.
13. Schallreuter K U, Wood J M. Free Radicals in Biology and Medicine 1989: 6: 519–532.
14. Schallreuter K U et al. J Invest Dermatol 1991: 97: 1081–1085.
15. Schallreuter-Wood K U et al. Arch Derm Res 1995: 6: 104–107.
16. Schallreuter K U. J Invest Dermatol Symp Proc 1997: 2: 1: 37–40.
17. Schallreuter K U et al. J Invest Dermatol 1998: 110–571.
18. Schallreuter K U et al. Biochim Biophys Acta 1998: 1382: 339–344.
19. Schallreuter K U. Pig Cell Res, Proceedings of the 8th European Pigment Cell Research Conference, Prague, Sept 1998 (in press).
20. Tobin D J, Thody A J. Exp Dermatol 1994: 3: 99–105.
21. Valverde P E et al. Nat Genet 1995: 11: 328–330.
22. Valverde P E et al. Pigment Cell Res 1996: 9: 77–84.
23. Wasterstrom S A. Scand Andid 1984: 1–40.
24. Wood J M, Schallreuter K U. Biochim Biophys Acta 1991: 1074: 378–385.
25. Wood J M et al. Biochem Biophys Res Commun 1995: 206: 480–485.
26. Stryer L. Biochemistry 3rd Edition, Freeman and Company 1987: 262.
27. Yohn J J et al. J Invest Dermatol 1991: 97: 405–409.

Viewpoint 2

Nature may often give us the clue for better understanding of what we humans do not yet know. For instance, the dark skin of Negroid people has a much better sun-protective property than the pale skin of Caucasians. Since the major difference of the skin between the two races is the melanin pigmentation, the melanogenesis cascade must be involved in combating the increased oxidative stress to the skin from the environment. Then how does the skin protection by melanin pigmentation occur, and which level of the melanogenesis cascade is responsible for this protection? Huge amounts of melanin pigments and degraded melanosomes are scattered freely on the skin surface. These melanin pigments are not enclosed in the membrane-bound organelle, but are embedded freely with keratin filaments in dead cells of horny-layer keratinocytes. In the Malpighian layer, keratinocytes receive melanin pigments, which are enclosed within membrane-bound melanosomes, from melanocyte dendrites, and these melanosomes lose their outer membrane and are degraded to release melanin pigments/dusts while ascending to the horny layer.

The hypothesis that melanin pigments and melanogenesis have an antioxidant function is interesting, but still needs to be further clarified at this stage. Dr Wood has given some valid arguments favouring antioxidant activity as the major functional role of melanin and melanogenesis. However, the antioxidant property of melanin pigments and their cytotoxic property may represent two faces of the same coin.

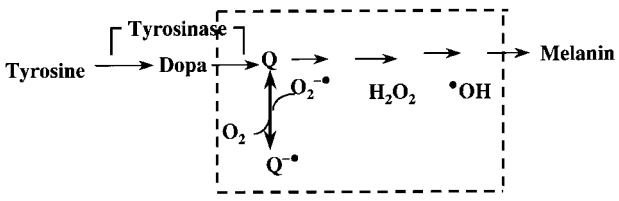

Figure 1. Oxidative stress during melanogenesis.

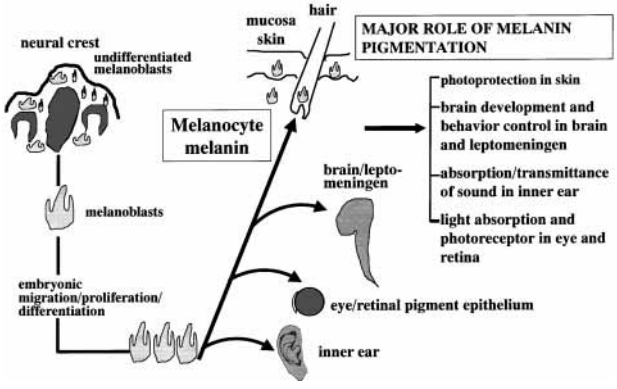

Figure 2. Embryonic development and major distribution of melanocytes, and possible functional role of melanin pigmentation.

While many studies have shown the free radical scavenging activity of melanin and melanin precursors (1), transfection of the tyrosinase gene into and induction of new melanin synthesis in several cell lines have demonstrated a similar cytotoxic effect of melanin and melanin precursors (2). Chemical modification of melanin precursors causes cytocidal or cytostatic effects to melanocytes through the interaction with tyrosinase (3, 4) (Fig. 1). One of the theories of UV-photocarcinogenesis actually implicates melanin-mediated oxidative stress as one of the early events in melanoma formation (5, 6).

Furthermore, the concept of melanin as an effective cellular antioxidant may not be consistent with its compartmentalization into membrane-bound organelles (melanosomes) (8). Assuming mitochondria are the major site of production of superoxide radicals, these molecules should escape mitochondrial SOD (MnSOD), should traverse cytosol-evading cytosolic SOD (CuZnSOD), and should finally be transported across the melanosomal membrane for the antioxidant action of melanin and its precursors to be affected. Furthermore, the cell contains several other antioxidant enzymes (catalase, glutathione peroxidase, etc.) and small molecular weight antioxidants (glutathione, ascorbic acid, alpha-tocopherol, etc.) which can also intercept the free radicals before reaching melanosomes. Also, most of the free radical species are highly reactive and short-lived. Thus, the confinement of melanin/melanogenesis to a membrane-bound organelle may not be consistent with an effective antioxidant role. However, an effective redox cascade ending up in melanin generation can not be ruled out.

Melanin pigments and related pigments are present not only in skin and hair but also other parts of vital organs (Fig. 2). The biological role of melanin pigments in the inner ear is still largely unknown. Little is known about the functional role of melanin pigments present in the leptomeninges, which cover the entire brain (7, 8). Importantly, melanocytes in these tissues are located in highly vascular areas along with blood vessels. The selective anatomic distribution and the differential functions of various melanin pigments are equally important and need to be clarified in the future. Recently, tyrosinase has been found in the brain (7). Production of melanin pigments may regulate the development of brain and may even control the behavior of individuals (cf. 9, 10). Whether or not this new biological role of melanin production is related to the antioxidant property of melanin pigments also requires further clarification.

Kowichi Jimbow
Dept of Dermatology
Sapporo University
Sapporo 060 Japan
E-mail jimbow@sapmed.ac.jp

References

1. Reszka K, Jimbow K. In: Funchs J, Packer L, eds. Current opinion in Dermatology. Marcel Dekker Inc 1993: 287–320.
2. Luo D et al. Exp Cell Res 1994: 213: 231–241.
3. Jimbow K et al. J Invest Dermatol 1993: 100: 231S–238S.
4. Riley PA et al. Euro J Cancer 1997: 33: 135–143.
5. Menon IA et al. Cancer Res 1983: 43: 3165–3169.
6. Jimbow K et al. J Invest Dermatol 1993: 100: 259S–268S.
7. Smythies J. Pro R Soc Lond B 1996: 263: 487–489.
8. Quevedo W C Jr et al. In: Fitzpatrick T B et al., eds. Dermatology in General Medicine, third edition. New York: McGraw Hill Book Comp, 1987: 224–258.
9. Sato S et al. Develop Dynamics 1997: 208: 363–374.
10. Tief K et al. Brain Res. Molecular Brain Res 1998: 53: 307–310.

Viewpoint 3

Melanins comprise a ubiquitous class of biological pigments found throughout the phylogenetic scale. Although consensus would indicate a photoprotective role for melanins, these amorphous polymeric pigments also offer protection from oxidative stress, even within the darkest recesses of the primate midbrain (1). An alternative hypothesis suggests that melanin's obvious contribution to skin colour evolved as a neutral trait without specific selection value, as described in viewpoint 1.

According to this hypothesis, melanin was selected for on the basis of its ion exchange properties, thereby providing an advantageous and heritable means of rapidly and efficiently maintaining calcium homeostasis. Certainly there is evidence to support this view. Melanins are capable of ion exchange (2), and melanin in the inner ear may actually serve to buffer intracellular calcium levels (3) through an ion exchange mechanism. Although there is evidence suggesting a role for melanin as a biological ion exchange polymer, the proposal that melanin's pigment properties are simply

an artifact of evolution, incidental to its natural selection as an ion exchange substrate, may require some additional thought.

If melanin's properties as a pigment were simply an artifact of evolution, one would probably not expect to find regulatory mechanisms in place that govern pigmentation. Neither should melanin function in a capacity that appears to depend upon its status as a pigment. Mammals exhibit seasonal variations in skin pigmentation (4) while many amphibian species (5) and teleost fish (6) are well known to modulate their pigmentation on time scales ranging from seconds to minutes. These observations would tend to refute the hypothesis that the pigment properties of melanin were ignored by natural selection.

Biological alterations in pigmentation are accommodated through elaborate regulatory mechanisms (7, 8), further suggesting that melanin's pigment properties were selected for during evolutionary history. Within an evolutionary paradigm, it would appear advantageous to select a molecule on the basis of multiple and seemingly independent physical and chemical properties. Efficiency is a characteristic of the natural selection process.

It is important to realize that cutaneous melanin's property as a highly visible pigment varying in concentration as a function of race and ethnicity may have had a chilling effect on efforts to elucidate many of its important functions (9). Hypotheses that suggest little selection value for cutaneous melanin on the basis of its pigment properties, appear understandable amid the recent atmosphere of "political correctness". Research directed at understanding the structure and function of neuromelanin (NM) may have particularly suffered in this climate.

NM is a macromolecular pigment found in dopaminergic neurons of the substantia nigra and other catecholaminergic brain cells within the locus coeruleus and the midbrain reticular formation (10, 11). NM may contribute to the defense against the oxidizing environment within these strategic brain loci (12). The concept that a neurochemical, no less a melanin, might be differentially distributed among human racial groups would have controversial implications. However, as Professor Wood points out, unlike melanin found in the hair, skin and eyes, there is little evidence that NM is synthesized through the action of tyrosinase. Cutaneous melanin's enzymatic synthesis by tyrosinase, possibly under differential post-translational regulation (13) as a function of racial groups, can explain how pigmentation varies within our species.

The fact that NM is neither a gene product nor the product of an enzyme reaction, needs to

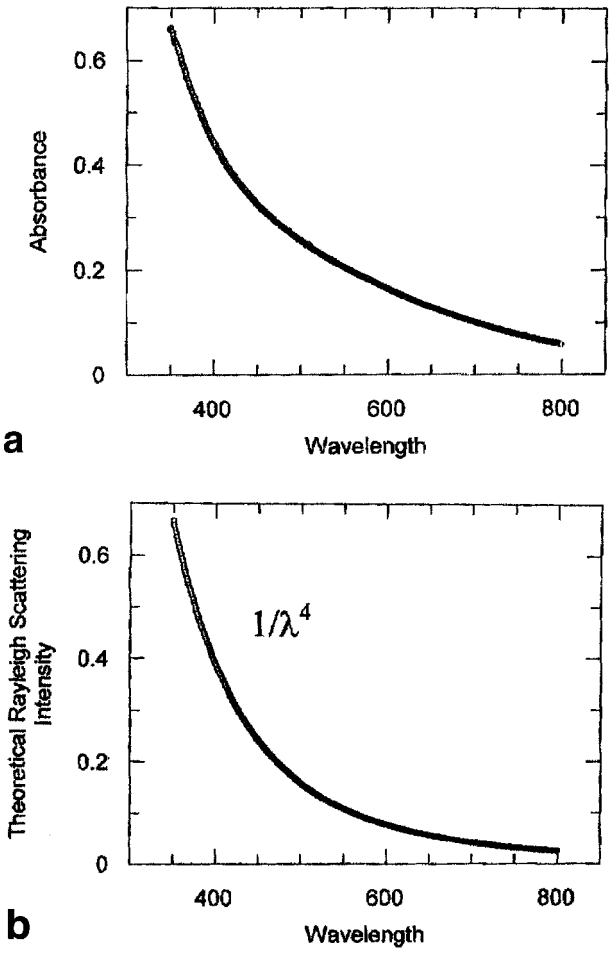

Figure 1. (a) Electronic absorbance spectrum of synthetic L-Dopa melanin (20 μg/ml in deionized water). (b). Theoretical relationship between Rayleigh light scattering intensity and wavelength.

be addressed since few molecules with significant physiological roles lack these fundamental means of regulation. It is now suggested that alterations in NM's aggregation state, as detected by Rayleigh light scattering, may provide a means through which its redox properties are regulated. This possibility is briefly developed in this viewpoint.

Although melanin appears to possess a pivotal role in mediating photoprotection of pigmented cells, it is paradoxical and frustrating that its photochemical and photophysical properties are studied usually with considerable difficulty. Some basic photophysical properties of melanin, however, can be observed in its absorption spectrum. When measured across visible wavelengths, melanin is found to possess a featureless spectral signature that increases monotonically with decreasing wavelength. A thoughtful analysis of these data

suggests that some similarity may exist between melanin's absorbance spectrum and the classical wavelength dependence of Rayleigh light scattering intensity (14) described by equation 1.

(1) $I=1/\lambda^4$.

The visible spectrum of synthetic L-dopa melanin is shown in Fig. 1a. Fig. 1b illustrates the theoretical relationship defined by equation 1. The similarity of these two curves is clear. Our laboratory is investigating the possibility that the visible spectrum of melanin represents a Rayleigh scattering envelope, under which are buried true absorbance bands. This is being explored through the use of resonance-enhanced Rayleigh light scattering. This technique takes advantage of the dramatic increase in Rayleigh scattering that can occur when observed near an absorbance band (15). Scanning the Rayleigh scattering spectrum of melanin may, in theory, have the capacity to identify regions of the Rayleigh scattering envelope under which true absorbance bands are located.

It is known that melanins can form supermolecular aggregates that vary in size as a function of pH and ionic strength (16, 17). Changes in aggregation state are often monitored through light scattering. Melanin aggregation accounts, in part, for the mechanisms attending the ability of some amphibian species to change skin color (18). The facile aggregation of synthetic L-dopa melanin at low pH is often used as a means of purification achieved through washing of acid-aggregated melanin.

We have recently reported that L-dopa melanin aggregates during several of its redox reactions (19). It also appears that melanin's redox environment modulates its aggregation state while its aggregation state can modulate its redox properties. This redox switching of aggregation state has been observed in a variety of molecules including cyanin dyes (20) and synthetic organometallic amphiphiles (21). It is tempting to speculate that the reciprocal relationship between melanin's aggregation state and redox properties may represent a mechanism through which its biological activity may be modulated

Clearly, the questions about the natural selection of melanin's ion exchange properties discussed in this viewpoint are similar to those that arise when we consider the possible functions and origin of NM, a neuronal pigment molecule, anatomically located where the sun simply does not shine. These and related questions will probably intrigue investigators for a long time to come. It is hoped that the fascinating theoretical issues surrounding melanin's surprising breadth of physical and chemical properties will continue to tempt new investigators to pick up the gauntlet and have a closer look at this intriguing molecule.

Jeffrey M. Tosk
Dept of Biology
La Sierra University
Riverside, CA 92515, USA
E-mail: jtosk@lasierra.edu

References

1. Enochs W S et al. J Neural Transm Park Dis Dement Sect 1994: 7: 83–100.
2. Sarna T et al. Science 1976: 192: 1132–1134.
3. Meyer zum Gottesberge A M. Pigment Cell Res 1988: 1: 238–249.
4. Bubenik G A, Bubenik A B. J Exp Zool 1985: 235: 387–395.
5. Miyashita Y et al. J Exp Zool 1996: 276: 125–131.
6. Groneveld D et al. Neuroendocrinol 1995: 7: 527–533.
7. Svensson S P et al. Pigment Cell Res 1997: 10: 395–400,
8. Furumura M et al. Proc Natl Acad Sci USA 1998: 95: 7374–7378.
9. Barr F E. Med Hypotheses 1983: 11: 1–139.
10. Lohr J B, Jeste D V. Acta Psychiatr Scand 1988: 77: 689–697.
11. Chan-Palay V, Asan E J. Comp Neurol 1989: 287: 357–372.
12. Korytowski W et al. Arch Biochem Biophys 1995: 319: 142–148.
13. Iozumi K et al. J Invest Dermatol 1993: 100: 806–811.
14. Anglister J, Steinberg I Z. Chem Phys 1983: 75: 443–452.
15. Pasternack R F, Collings P J. Science 1995: 269: 935–939.
16. Enochs W S et al. Invest Radiol 1989: 24: 794–804.
17. Bridelli M G. Biophys Chem 1998: 73: 227–239.
18. Rogers S L, Gelfand V I. Curr Biol 1998: 8: 161–164.
19. Tosk J M et al. Abstract 400.10, Society for Neuroscience, 28th Annual Meeting, Los Angeles, CA Nov. 7–12, 1998.
20. Lenhard J R, Hein B R. J Phys Chem 1996: 100: 17287–17296.
21. Munoz S, Gokel G W. Inorganica Chim Acta 1996: 250: 59–67.

Commentary 1

The generation of melanin in the epidermis appears to impart various protective mechanisms for maintaining healthy skin. These include protecting the skin from both environmental assaults primarily through ultraviolet (UV) light and cellular damage, primarily through reactive oxygen species (ROS), and by modulating different physical properties of the skin. The UV absorptive characteristics of melanin, both in the melanocyte where it is synthesized and in the keratinocyte where it is transferred to and preferentially localized over the apical pole of the nucleus, clearly impede DNA damage and the subsequent development of epidermal cancers and melanomas. Dark-skinned individuals with an increased quantity of eumelanin transferred to keratinocytes are less likely to develop skin cancers than fair-skinned individuals, especially when the latter group is UV challenged after relocalizing towards the equator (1, 2).

This documented protection is quite amazing in light of the fact that the sunlight protection factor (SPF) of melanin is relatively limited (3), as described in Viewpoint 1. One would question whether using commercially available sun blocks with SPF values over 10 is of any significant value. In fact, blocking out more than 96% of the UV, as accomplished by sun screens with SPF >25, could impede the sun-induced upregulation of cutaneous melanization that could provide an effective protection for subsequent UV exposure.

Eventually, pigmented melanosomes that are transferred to the basal and lower stratum spinosum keratinocytes become degraded. By the time the keratinocytes migrate through the stratum granulosum and into the stratum corneum, melanosomes are no longer apparent as distinct organelles, except occasionally in very dark-skinned individuals. However, melanin (occasionally referred to in the literature as "melanin dust") is present in the superficial epidermis. If the stratum corneum is removed it has a dark hue to it, more prominently so when obtained from dark skinned individuals.

What are the functions of the retained melanin polymers in the more superficial epidermal layers? Do they still effectively absorb UV light, scavenge oxygen radicals, and/or influence physical parameters of the skin? Also, melanins can bind various chemicals, metal ions, and drugs (4). Perhaps, melanins at the interface of the skin and the environment effectively trap industrial and pathological chemicals and toxins that are absorbed through the skin, and quickly return them to the environment during desquamation.

In addition to the UV protection melanin affords to dark skin as opposed to light skin, the degree of melanization also correlates with differential physical properties of skin (5) and hair (6). For example, darkly pigmented skin exhibits reduced transcutaneous penetration, whereas lightly pigmented skin exhibits a stronger resistance to water barrier damage. Pertaining to hair pigmentation, the loss of melanin in the hair shaft correlates with the inability of the hair to hold either a temporary or permanent set. The concept that melanin in the epidermis affects physical properties and possibly pathologic conditions of the skin certainly needs to be investigated further.

Another benefit melanin provides to the epidermis is that of a scavenger and/or neutralizer of superoxide anion radicals (O_2°). Again, mechanisms by which melanin is involved in neutralizing reactive oxygen species (ROS) to prevent damage of cellular proteins, lipids and nucleic acids are well presented in Viewpoints 1–3. Melanin putatively protects the melanocyte from its own ROS intermediate indoles and quinones of melanin biosynthesis, specifically those melanocytes that permanently reside in the stratum basale through the life of the individual. In addition, melanin putatively protects the recipient keratinocyte from the potential ROS generated in this very metabolically active cell.

However, despite the clear experimental evidence that melanins are a free radical neutralizing polymer, the benefit of this protection is not apparent in the skin. The health of the skin in general, and specific, differential consequences of ROS-induced cellular damage between dark- and light-skinned individuals, in concert with the melanin content, have not yet been demonstrated in the published literature. Melanocytes are lost from the epidermis with age (7, 8). However, whether melanocyte loss is accelerated or ultimately increased relatively in light-skinned individuals has not been documented. Also undocumented is whether keratinocytes from light-skinned individuals are more susceptible to ROS damage or not. If melanin in the recipient keratinocyte does indeed protect it from ROS damage, this may be of fairly little physiological consequence, due to the fact that keratinocytes may rapidly undergo terminal differentiation and desquamation before cytologic damage by ROS can accumulate.

However, a correlation between the melanin sta-

tus and cell protection has been demonstrated in ocular tissue. The age-related cell death of various neuronal cells in the retina is more pronounced in albino rats than in pigmented rats (9). However, whether this protection is the result of melanin combating the effects of UV or ROS is unclear. Extracutaneous melanin, specifically eumelanin synthesized by melanocytes of the eye, is particularly interesting when exploring the functional properties of melanins.

There are two embryonically derived categories of pigment cells in the eye. One type comprises the retinal pigment epithelium (RPE), which is juxtaposed over the photoreceptor cell layer of the retina and is derived directly from the neuroectoderm that forms the eye orbit. The second type is localized in the mesenchyme of the uveal tract, composed of the choroid and ciliary bodies, and the iris, and is derived from neural crest cells, as are the cutaneous melanocytes. Melanin is synthesized in the RPE early during embryogenesis of the eye, where it appears to regulate in part the correct development of the optic tract to the visual centers of the brain. In higher vertebrates and primates, half of the optic nerve fibers traverse from each eye to the visual cortex on the ipsilateral side of the brain, whereas the other half crosses at the optic chiasma to the contralateral side of the brain. This segregation allows for the assessment of depth perception and relative movement of objects by organism with frontal vision.

In animals and individuals with any of the genetic forms of albinism, the ratio of crossed fibers is shifted towards the ipsilateral pathway resulting in visual acuity problems (10, 11). Since in all forms of albinism the embryonic differentiation of the melanocyte is normal, and only the ability of the melanocytes to make melanin is reduced or prevented, it has been hypothesized that the melanin polymers generated by the ocular melanocytes regulate this neuronal establishment. The molecular mechanism responsible for this has yet to be assessed.

As noted in Viewpoints 1 and 3, most of the melanin synthesized in the brain is of the neuromelanin type derived from dopamine. However, there is a subpopulation of neural crest-derived melanocytes that synthesize eumelanin within melanosomes that reside in the leptomeninges overlying the ventrolateral surfaces of the medulla oblongata and the upper cervical cord (12). The function of these evolutionarily retained melanocytes is illusive. One could speculate that the melanin produced in this localized area of the brain might have a specific regulatory role in neuronal development as described earlier in the eye.

Raymond E. Boissy
Dept of Dermatology
University of Cincinnati College of Medicine
Cincinnati, OH 45267, USA
E-mail: boissyre@email.uc.edu

References

1. Kricker A et al. Int J Cancer 1991: 48: 650–662.
2. English D R et al. Int J Cancer 1998: 67: 628–634.
3. Kaidbey K H, Kligman A M. Arch Dermatol 1978: 114: 46–48.
4. Larsson B S. The toxicology and pharmacology of melanins. In: Nordlund J J et al., eds. The Pigmentary System: Physiology and Pathophysiology, New York: Oxford University Press, 1998, 373–389.
5. Berardesca E, Maibach H. J Am Acad Dermatol 1996: 34: 667–672.
6. Wickett R R. Similarities and differences in physical properties of fray and pigmented hair (abst). J Invest Dermatol 1999: (in press).
7. Quevedo W C Jr et al. J Invest Dermatol 1969: 52: 287–290.
8. Ortonne J P. Br J Dermatol 1990: 122: 21–28.
9. Weisse I et al. Elns Eye Toxic Res 1990: 7: 717–739.
10. Guillery R W. Sci Am 1974: 230: 44–55.
11. Creel D et al. Science 1978: 201: 931–933.
12. Goldgeier M H et al. J Invest Dermatol 1984: 82: 235–238.

Commentary 2

It is now recognized that precursors to melanin function as substrates for specific melanogenic enzymes and as regulators of non-melanogenesis-associated functions (1–3). For example, L-tyrosine, L-dopa or their derivatives can regulate expression, assembly and functions of the melanogenic apparatus, the expression of MSH receptors and the phophorylation/dephosphorylation cascade in melanocytes (1–7). Furthermore, melanogenesis can regulate the energy-yielding metabolism of

pigment cells by switching oxidative to anaerobic glycolysis, altering the NAD/NADH and NADP/NADPH ratio, or by stimulating the pentose phosphate pathway (cf. 1–3). Therefore, it has been proposed that L-dopa, a product of the enzymatic oxidation of L-tyrosine, can act as a "second messenger" of the pathway stimulating cellular metabolism, to then undergo rapid inactivation by the dopa oxidase activity of tyrosinase (5). Other short-lived products of L-dopa metabolism such as cysteinyldopa, dihydroxyindole (DHI) or DHI carboxylic acid (DHICA) may also act as additional "second messengers" (2).

Thus, the process of melanogenesis would act as a regulatory and self-regulating pathway through the generation and inactivation of biologically active molecules, with melanin pigment as the final product of this process. Because of the required oxygen consumption, melanogenesis may result in cellular hypoxia, which would affect the energy-yielding metabolism and may increase the resistance of melanocytes to ionizing radiation.

In intact skin, melanogenesis takes place in melanosomes, organelles which are then transferred from melanocytes to epidermal keratinocytes or to hair bulb keratinocytes during the anagen stage of the hair cycle (2, 8). We have proposed that melanized or melanizing melanosomes can act as "regulatory packages" that are sent to keratinocytes in order to regulate their differentiation level and metabolic status (2). This is based on the effects discussed above on cellular metabolism, and on the intrinsic calcium buffering properties of melanin, as well as on its ability to reversibly bind bioregulatory molecules such as prostaglandins. In addition, melanin is a well-known protector against the damage induced by solar radiation.

Of further interest is the effect of melanogenesis on immune functions. Thus, L-dopa and products of its oxidation can act as immunosuppressors by inhibiting T and B cell activities, whereas lymphocytes themselves can produce L-dopa (reviewed in ref. 9). Therefore, we have suggested that pigmented melanocytes can specifically alter functions of the local skin immune system by releasing intermediates of melanogenesis (2, 9). In the case of melanotic melanoma cells, these might evade immune destruction by releasing immunosuppressive intermediates of melanogenesis, thus contributing to tumor progression and impairing the effectiveness of immunotherapy (9).

In conclusion, the process of melanogenesis, and melanin itself, may serve very complex biological functions and may alter cellular metabolism and differentiation as well as immune functions in restricted skin compartments. Future research should clarify more systematically the precise bioregulatory role of intermediates of melanogenesis, such as L-tyrosine, L-dopa, cysteinyldopa, DHI and DHICA. The primary effort in this area may best be focused on the identification, characterization and cloning of receptors or regulatory proteins that are activated or inhibited by these compounds.

Andrzej Slominski
Department of Pathology
Loyola University Medical Center
Maywood, IL 60153, USA
E-mail: aslomin@wpo.it.luc.edu

Jacobo Wortsman
Department of Medicine
Southern Illinois University
Springfield, IL, USA

References

1. Slominski A, Paus R. J Theor Biol 1990: 143: 123–138.
2. Slominski A et al. J Theor Biol 1993: 164: 103–120.
3. Slominski A, Paus R. Moll Cell Endocrinol 1994: 99: C7–C11.
4. Slominski A et al. J Cell Sci 1988: 89: 287–296.
5. Slominski A et al. Pigment Cell Res 1989: 2: 109–116.
6. Slominski A et al. Biosci Rep 1989: 9: 579–587.
7. Slominski A, Friedrich T. Pigment Cell Res 1992: 5: 396–399.
8. Slominski A, Paus R. J Invest Dermatol 1993: 101: 90S–97S.
9. Slominski A et al. Anticancer Res 1998: 18: 3709–3716.

Commentary 3

According to "Viewpoint 1", melanins are synthesized to protect against the toxicity of some reactive oxygen species (ROS) which result from mitochondrial respiration. Indeed, it is only aerobic organisms, even those as simple as *Turbellaria* (e.g. *Polycellis nigra;* 1), which exhibit full melanization.

However, many other aerobic invertebrates (e.g.

various *Hydrozoa*) do not produce melanins (1). Some vertebrates, for example amphibia and fish (e.g. *Anoptichtys jordani*) living in well-oxygenated and dark environments such as caves, are almost devoid of these pigments. What is more, even among the anaerobic parasites – *Cestoda,* a few species are able to produce melanins (e.g. *Tentacularia coryphaenae* – a fish parasite [1, 2]). In general, neither the presence of melanin in aerobic organisms, nor its absence in anaerobic organisms are a rule. Adaptation to aerobic metabolism, therefore, is clearly a secondary reason for the synthesis of melanins.

Among the invertebrates there are examples of a connection between melanization and immunity; for example some insects react to the appearance of a parasite by encapsulation of the intruder and by melanization (3, 4). This may be a defensive reaction targeting the parasite, or simply a means of protecting the surrounding tissues against ROS produced during the struggle. The invader may use the same ability against the host's defence: the only melanized part of the body of *Tentacularia coryphaenae* is the scolex, i.e. the organ which directly invades and irritates the host tissue.

Therefore, an alternative hypothesis may be proposed: melanins are an adaptation not just to the presence of, but to an *excess* of ROS. Other examples supporting this view are *Azotobacter chorococcum* (5) and *Rhizobium* sp. (6), i.e. nitrogen-fixing bacteria where melanogenesis is believed to ensure anaerobic conditions necessary to assimilate atmospheric nitrogen.

Light is an important factor influencing *in vivo* melanogenesis (7), and a common constituent of the environment of numerous pigmented organisms: "Tanning" may even be observed in the cytoplasm of the very simple Eukaryote, *Physarum nudum* (*Myxomycetes*; 8). Melanophores can be found even in sea *Diblastica* (*Ctenophora*). In leeches (*Hirudinea*), fully-developed melanophore dendrites are organized in a net embedded in parenchyma (1). *Arthropoda* exhibit very rich patterns of cuticular coloration (9), and the melanophores of *Cephalopoda* are responsible for rapid and expressive changes in body colour (1). On the contrary, organisms living without light are often devoid of melanins (e.g. the common earthworm *Lumbricus terrestris*, and *Anoptichtys jordani,* as mentioned earlier).

However, besides the explanation suggested in "Viewpoint 1", there is another biological reason for why it is useful to produce melanin in the presence of light. By means of pigmentation, all these animals advertise or conceal their presence, and even, in the case of *Cephalopoda,* express mood. Thus, in the animal kingdom, the question "to produce, or not to produce melanin" probably boils down to the much simpler formula: "to be, or not to be visible". Signalling and camouflage, therefore, may well be the true primary functions of melanogenesis.

Przemyslaw M. Plonka
Department of Biophysics
The Jan Zurzycki Institute
of Molecular Biology
Jagiellonian University
31–120 Krakow, Poland
E-mail: Mieszko@mol.uj.edu.pl

References

1. Sembrat K. Comparative Histology of Animals. Vol. II. Warsaw: Polish Scientific Publishers, 1981.
2. Carvajal J et al. J Parasitol 1976: 62: 70–77.
3. Marmaras V J et al. Arch Insect Biochem Physiol 1996: 31: 119–133.
4. Richman A, Kafatos F C. Curr Opin Immunol 1996: 8: 14–19.
5. Shivprasad S, Page W J. Appl Environ Microbiol 1989: 55: 1811–1817.
6. Cubo M T et al. Appl Environ Microbiol 1988: 54: 1812–1817.
7. Schallreuter et al. Exp Dermatol 1998: 7: 143–150.
8. Plonka P M, Rakoczy L. Curr Top Biophys 1997: 21(1): 83–86.
9. Sugumaran M. FEBS Lett 1991: 293: 4–10.

Commentary 4

Melanins and closely related humic acids (soil and water allomelanins) are the most primitive dark paramagnetic biopolymers. Their unusual biophysicochemical properties as well as their preferential distribution in surface layers of organisms or ecosystems may account for previously unexplained phenomena. The role of melanins as oxygen radical scavengers (traps), UV-radiation filters, and of polymeric buffers for ions of transition metals, for calcium and for catecholamines is well-covered in Viewpoint 1. Dr Wood's original and holistic approach follows the integrative framework proposed 15 years ago by Frank Barr (1), and seems to best reflect the multifunctional properties of the mel-

anin biopolymer. In addition to discussing the classical antioxidative (protective) functions of epidermal eumelanin and neuromelanin as reactive oxygen species–trapping polymers, Wood proposes the novel concept that the skin colour may have evolved secondarily as a side effect through natural selection principles in tropical versus temperate climates. This interesting idea stems from Wood's integrative approach, which carefully considers the subtle link existing between calcium homeostasis and melanogenesis. However, in view of the unusual complexity of the melanin polymer, and in the face of our still limited knowledge about melanin structure, properties and interactions, this may best be considered as a working hypothesis requiring further research and verification.

Perhaps, additional light may be shed on the biological functions of melanin if we turn our attention to certain aspects of research that may be less familiar to dermatologists. The first is the bioenergetics of hornets and wasps. Black melanin and yellow pteridin strips (rings) in the insect's abdomen can function as photosemiconductor junctions, which produce a photovoltaic gradient when illuminated (2). It has been speculated that the voltage gradient can assist phosphorylation, or that the melanin-pteridin junctions act as species-specific light-emitting diodes.

The second aspect is mimicry, i.e. the rapid change in the amount, kind (?) and distribution pattern of melanocytes or melanosomes in the epidermis of some insects, making them indistinguishable from the environment. This function is of vital importance for survival, and cannot be a mere side effect of evolutionary selection.

A third, relatively new aspect is the generation of electronically excited states during the formation and degradation of melanins. *In vitro*, the enzymatic synthesis of eumelanins and pheomelanins does not produce light (chemiluminescence) with the quantum yield $>10^{-13}$, while autooxidation is accompanied by weak light emission (3, 4). However, oxidative degradation of melanins and humic acids stimulated by reactive oxygen species or radiation, which is associated with the splitting of their aromatic rings, always produces weak chemiluminescence (5–7). Therefore, the role of electronically excited states and of photons generated *in situ* within a melanocyte is the subject of much speculation (7, 8), but should not be underestimated in the light of our knowledge about "photobiochemistry without light" (9).

Finally, the role of melanins as polymeric buffers for transition metals is now obscured by recent findings which indicate that the association of melanin with metal ions drastically changes its dielectric and electric properties (10). However, the question how these electric or paramagnetic properties might be correlated with the physiological functions of melanin remains unanswered. In conclusion, in order to generate a complete picture of melanin functions, these additional aspects deserve systematic exploration in the context of the dynamic web of melanin interactions, as lucidly depicted by Wood.

Janusz Slawinski
Inst. of Chemistry & Technical Electrochemistry
Poznan University of Technology
PL-60965 Poznan, Poland
E-mail: slawinsk@sol.put.poznan.pl

References

1. Barr F. Med Hypotheses 1983: 11: 1–140.
2. Rosenzweig E et al. Physiol Chem Phys & Med NMR. 1985: 17: 435–449.
3. Slawinska D, Slawinski J. Pigment Cell Res 1987: 1: 171–175.
4. Villablanca M et al. J Biolumin Chemilumin 1989: 3: 189–190.
5. Slawinska D, Slawinski J. Physiol Chem Phys 1982: 14: 363–374.
6. Polewski K, Slawinska D. Physiol Chem Phys & Med NMR 1991: 23: 125–132.
7. Górski Z et al. Curr Top Biophys 1996: 20: 128–133.
8. Villablanca M. An Acad Brasil Cienc 1986: 58: 4–8.
9. Cilento G. Experientia 1988: 44: 572–576.
10. Osak W et al. J Biol Phys 1995: 21: 51–65.