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Editorial Office: G. Ghanem (Editor), C. Meunier, R. Morandini (Production Team), Laboratory of Oncology and Experimental Surgery (L.O.C.E.), Université Libre de Bruxelles, Institut J. Bordet, Rue Héger-Bordet 1, B - 1000 Brussels, Belgium.
Phone: 32-2-535.35.46 Fax: 32-2-534.95.50 E-Mail: gghanem@resulb.ulb.ac.be

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LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

Zinc in pigmented cells and structures, interactions and possible roles

Jan Borovanský
2nd Department of Biochemistry, 1st Faculty of Medicine,
Charles University, 128 53 Prague 2, Czech Republic
Reprinted with permission from Dr Milan Spala, Editor-in-Chief of Sbornik lékařsky Journal
Zinek v pigmentovych buňkách a strukturách -jeho interakce a možné role. *Sborn. lék. Vol. 95 (1994) No. 4, p. 309-320.*

SUMMARY: Zinc is a feature trace element of pigment cells and tissues. Organelles, in which melanin is synthesized and stored, i.e. melanosomes, represent a zinc reservoir at the subcellular level. In order to understand function of metals in tissues, cells and their constituents, knowledge is needed on metal interactions with intracellular targets. The possible zinc ligands in pigment cells include melanin, metallothionein, melanotransferrin, B700 and related proteins, ferritin, zinc enzymes and low molecular weight ligands. Areas of a special interest in relation of pigment cells and structures to zinc - such as zinc effect on melanogenesis, zinc excretion and buffering by melanosomes, zinc function in free radical processes as well as zinc role in melanomas - have been reviewed. High level of zinc in pigment cells may indicate a physiological defense against the potential danger of oxidative stress.

A large number of natural pigments is associated with metals, namely with iron, copper, manganese or vanadium [7]. Feature trace element of melanoprotein pigments is zinc.

1. ZINC IN PIGMENT CELLS AND TISSUES

The strikingly high zinc level in pigment tissues was first noticed in pigment structures of eye [17,19,36,58,59,85] and later demonstrated in pigmented normal [45] and tumour tissues [46,58,65]; high level of zinc was demonstrated also in pigmented regions of human brains [29,48].
Experiments with radioactive ^{65}Zn revealed high uptake of zinc into murine tumours - Cloudman S91 melanoma [65], B16 melanoma [10,75] and Harding-Passey melanoma [10]. Newsome and Rothman [63] described the ability of human retinal pigment epithelial cells in vitro to accumulate and retain zinc, later study of the same group verified *in vivo* that pigment eye tissues of humans and primates took up and retained zinc [62]. Dencker and Tjälve [28] mentioned retention of ^{65}Zn in hair of pigmented C57BL6 mice.

2. ZINC IN MELANOSOMES

With the development of cell fractination techniques it became obvious that at the subcellular level zinc was deposited especially in melanosomes [41,86,90]. Our comparative studies demonstrated that melanosomes represent unique subcellular storehouses of zinc because the Zn concentration in the isolated organelles exceeded that in the whole original pigment tissue 3-5fold [12,46] - Tab. 1.

Table 1 - Zinc concentration in pigment tissues and in melanosomes isolated from them

SPECIMEN	TISSUE	MELANOSOME
bovine uvea	138.4 ± 2.3	598.0 ± 4.2
human hair	158.0 ± 23.2	664.0 ± 376.6
Harding-Passey mouse melanoma	75.5 ± 1.8	383.3 ± 2.2
horse melanoma	112.0 ± 1.9	544.3 ± 4.1
human melanoma	181.1 ± 7.5	612.1 ± 5.2
Bomirski hamster melanoma (line Ma)	185.0	417.1

The result s are expressed in μ Zn/g dry sample (x ± SD). Compiled from [12, 45, 46]

The initial data derived from colorimetric measurements were later confirmed by modern techniques such as neutron activation analysis [78] or mass spectrometry [92] but there still has persisted a question if the zinc was not absorbed artificially by melanosomes during isolation procedure. Only X-ray microanalysis of melanosomes *in situ* brought a conclusive evidence for the presence of zinc in trout skin melanosomes [72], in melanosomes of inner ear and uveal tract [60], in retinal and choroidal pig melanosomes [82] and in melanosomes of human retinal pigment epithelium [94]. Only Takaya [91] using X-ray microanalysis found neither zinc nor copper in hair melanosomes.

The presence of zinc was demonstrated also in the pigment extracted by a mild procedure from *substantia nigra* of human brains [101]. If zinc is the abundant trace element of melanosomes (*e.g.* its concentration in human hair melanosomes is the highest Zn concentration attained in a structural element of human body), the next question striking mind is where and why it is localized in these organelles.

Zinc-melanin and zinc-protein interactions can be expected to occur in melanosomes. What is the distribution of zinc between melanin and protein moieties of melanosomes has not been clearly defined because only a few studies have addressed the cardinal question of zinc distribution within melanosomes.

Procházková et al. [77] having digested the isolated melanosomes of Harding-Passey mouse melanoma with chymotrypsin separated the proteins electrophoretically on agar and studied by neutron activation analysis the Zn distribution among protein fractions. All the protein fractions displayed the presence of zinc, but a colourless protein band with the highest anodic mobility contained more than a half of the zinc associated with melanosomal proteins.

Zinc pool of melanosomes seems to be quite labile: It was possible to remove all hot Zn by 5 day exchange diffusion against 1mmol/l $ZnCl_2$ from B16 mouse melanoma melanosomes labelled with ^{65}Zn *in vivo* [10,11]. Treatment with 0.5 mmol/l acetic acid released 100% of radioactive zinc from the melanosomes as well. If the B16 melanosome acetic acid supernatant was passed over a Biogel P-2 column, 55% of ^{65}Zn was eluted in the void volume indicating a bound form of ^{65}Zn, less than 50% of ^{65}Zn was eluted in the salt volume (= free ^{65}Zn) [11]. When the supernatant of SDS-treated B16 mouse melanoma labelled melanosomes was passed over an Ultrogel AcA54 column, ^{65}Zn was eluted in a fraction of a molecular weight in the region 15,00 - 18,00 [11].

There have been also observations suggesting indirectly the importance of non-pigment moieties of melanosomes for zinc binding. To this category falls e.g. a report of Shibata et al.[87] showing that Zn level was higher in premelanosomes than in melanosomes of Green's hamster melanoma.

3. NATURALLY OCCURRING ZINC LIGANDS IN PIGMENT CELLS AND STRUCTURES

In order to understand the function of metals in living systems, knowledge is needed on the biochemical basis of metal interactions with intracellular targets. The balance between essentiality and toxicity of metals can be regulated by specific binding sites for metals and hence knowledge concerning intracellular biochemical speciation is of importance.

3.1. Melanin

Melanin behaves as a natural cation exchange material [97] and is therefore able to incorporate various ions both *in vitro* and *in vivo* [23]. The analysis of the affinity of synthetic and natural melanins for inorganic ions showed interestingly that zinc was on the lower scale of ionic affinity [74]. Detailed study on binding capacity of metal ions to synthetic dopa melanins demonstrated that two classes of independent binding sites participated in the interactions of cations with dopa-melanin, with association constants for Zn $K_1=5.87 \times 10^5 mol^{-1}$, $K_2=4.85 \times 10^3 mol^{-1}$ [25].

Situation *in vivo* is expected to be more complicated: *1*) Competition between various metal ions for binding sites on melanin can influence the binding parameters as evidenced by model experiments *in vitro* [9]. *2*) Melanin pigments in melanosomes *in vivo* are always associated with a protein moiety which can also influence metal ion - melanin interactions. Among various metals only zinc was found in a higher amount in the melanin-human albumin-Zn complexes, unlike Mn, Cu and Fe binding of which decreased in the presence of albumin [3]; recently the binding capacity of melanoprotein isolated from bovine eyes for Zn^{2+} was found to be by 10 - 20 % lower compared with that of protein-free melanins [2]. The importance of protein in melanin-protein complexes for zinc binding was emphasized already by Bowness and Morton [18] but their results are difficult to interpret due to the usage of phosphate buffers in their experiments.

3.2. Metallothionein

Metallothionein is an important intracellular ligand for zinc and copper as well as for some other transition metals [70].It is believed to be involved in the homeostatic control of Zn absorption, in cellular detoxification, in the control of differentiation and in direct activation of Zn-dependent enzymes [21,31,79].

The metabolic and growth demands of neoplastic tissue may make tumours the predominant site of Zn uptake [10,70,96] which is accompanied by hypozincemia [26,31,70,89]. This is a result of a number of factors, some unrelated to tumour. Hypozincemia has been also recorded in melanoma patients [47]. Further zinc redistribution during tumour-related stress can be induced by a rise in the amount of hepatic metallothionein [70,93]. Some authors suppose [70] that release of Zn2+ from lysing tumour cells may subsequently enable hepatic metallothionen synthesis to proceed.

Quantification of the copper-binding compounds in equine melanoma tumours revealed that as much as 50 - 60 % of total tissue copper was associated with metallothionein whereas tyrosinase and Cu_2Zn_2-superoxide dismutase accounted for appr. 2% of total copper [56]. The same situation is assumed for human melanoma tissue. Zn binds less strongly than Cu to metallothionein and can, therefore, be readily displaced by Cu [21] but Krauter *et al.* [56] found equimolar concentrations of zinc and copper in their samples which suggested that metallothionein might be the major protein ligand for zinc in pigment cells.

This would be in accord with the generally accepted concept of metallothionein as an autoregulated intracellular zinc (and copper) buffer [79] establishing intracellular steady state kinetics for Zn and Cu levels. As for pigment cells there have been only rare reports dealing with a specific role of metallothionein in these types of cells: Koropatnick and Pearson [55] studied B16 melanoma cells with low and high metallothionein constitutive expression and concluded that metallothionein was associated with cisplatin resistance. Oliver et al. [66] demonstrated that induction of metallothinein synthesis in human retinal pigment epithelial cells was correlated with an increased capacity for ^{65}Zn

uptake into cultured cells.

Zinc bound to metallothionein is released after degradation of the metallothionein protein in lysosomes (unlike the fate of Cu-metallothionein which is different) [79], hence lysosomes may be involved in the accumulation of zinc [84]. If we accept the more and more common opinion that melanosomes are related to lysosomes [88,102], this mechanism would offer an explanation for high Zn level in melanosomes.

3.3. Melanotransferrin

Melanotransferrin, also known as the tumour-associated antigen p^{97}, is a monomeric glycoprotein expressed at high levels in most human melanomas but present in only trace amounts in normal adult tissues [22]. The comparison of the primary structure of p^{97} with that of other members of the transferrin superfamily revealed a Zn-binding concensus sequence found in metallopeptidases within the N-terminal lobe and in the C-terminal lobe a glutamic acid residue capable of completing a potential thermolysin-like Zn binding site [37]. Thus p97 may have a Zn-binding potential, unique amongst the transferrin superfamily. In contrast to other transferrins, melanotransferrin binds only one Fe^{3+} ion per molecule [5]. Functional consequences for melanoma cells with high p97 expression in melanoma cells have not so far been investigated.

3.4. B700 and related proteins

B700 protein is the major protein of the murine melanoma cell's melanosomal membrane; it is also present in the membrane of other cytoplasmic organelles as well as in the plasma membrane [44]. There are related proteins in melanomas of other species [39]. It has become obvious that the B700 protein is part of the serum albumin family of proteins [38]. A number of studies underscored the importance of controlling the relative concentrations of Zn and its ligands in Zn transport kinetic research and suggested that varying their concentrations might be a method of regulating the distribution of Zn into specific cells and tissues [8]. Albumin belongs to Zn ligands with physiologically high Zn affinity (circa 10^7) [1,40]. There has been no information on the B700 affinity for zinc. However, if it maintained the Zn-ligand affinity typical of serum albumin, it would become another hot candidate to explain Zn presence both in melanosomes and pigment cells.

3.5. Ferritin

Ferritin is a "fashionable" molecule because it can be engaged in the deactivation of increased iron load. In the *substantia nigra* the disbalance between iron and transferrin levels has been suspected from triggering free radical damage in Parkinson's disease [29].

It is less known that ferritin may fulfill also zinc-sequestering and -dispensing tasks. It has been postulated that ferritin may serve as the initial chelator for Zn^{2+} (and other metal ions) prior to the synthesis of metallothionein is initiated as the second line of defence [76]. No data on the concentration of ferritin in pigment cells have been available, though.

3.6. Zn-enzymes

The magnitude of the stability constants of metal binding proteins varies quite widely and has served to differentiate operationally between two classes, metalloproteins and metal-protein complexes [95] with firm and loose metal binding, respectively. Zinc containing enzymes fall in both groups.

There has been no Zn enzyme described the concentration of which in pigment tissues would be profoundly different from other tissues. It is only possible to mention high α-D-mannosidase expression in melanomas [32], (this enzyme has been suggested as a possible general indicator of Zn status [34]), and early papers emphasizing the importance of carbonic anhydrase to explain high Zn level in eye pigment tissues [36,59].

The marker enzyme of melanogenesis - tyrosinase - belongs to copper-containing proteins. It would be interesting to ascertain whether the recently discovered tyrosinase-related proteins are metalloenzymes and if so, what is their metal dependence.

3.7. Binding of zinc to low-molecular-weight ligands

Metal ion interactions with low-molecular-weight ligands *in vivo* are extraordinarily difficult to study due to the very low concentrations which are involved and due to the labile nature of most such associations. Our present knowledge about the chemical binding which may, or may not, take place between zinc and low-molecular-weight agents has had to be inferred largely from computer simulations of the equilibria which are thought to dominate the low-molecular-weight fraction of the metal ion [21]. These studies have demonstrated that *e.g.* in blood binding is clearly dominated by cysteinate with histidine acting as the other important coordinating partner [21,40]. Reduced glutathionate seems likely to supersede cysteinate inside most, if not all cells [21]. The presence of Zn cysteinate was cytochemically confirmed in cat *tapetum lucidum* rod-shaped paraplasmic inclusions considered by some authors as melanosomes [53]. ^{1}H and ^{13}C NMR studies revealed that Zn^{2+} binds also with oxidized glutathione in aqueous medium with 1:1 stoichiometry [73]. Taking into account a significant role of glutathione for pigment cell metabolism [6], Zn-glutathione complexes may make the metabolic relations still more complex.

In pigment cells zinc - dopa interactions are also to be expected since L-dopa can bind zinc using its orthophenolic groups [51].

According to the prevailing opinion the small Zn^{2+}-species are involved in processes which exploit their kinetic advantages over the complex formed by proteins. For the most part, these involve transport to or through membranes and exchange between high-molecular-weight species [21] (Fig.1).

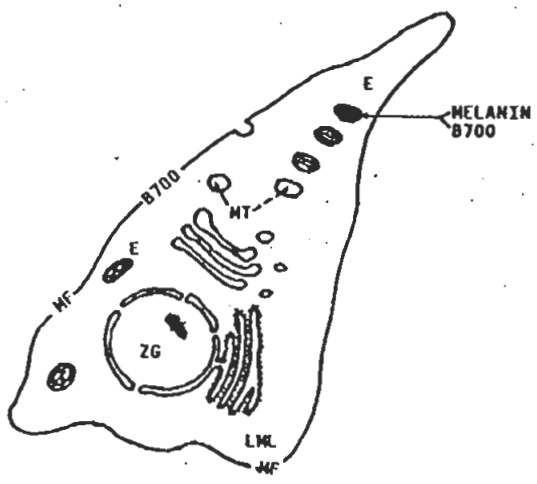

Fig. 1 - Points of special interest in zinc relation to pigment cells and structures.
B700 = B700 and related proteins, *E* = zinc enzymes, *MF* = melanotransferrin,
MT = metallothionein, *LML* = low-molecular weight-Zn ligands
ZG = zinc gene regulatory proteins.

4. FUNCTIONS OF ZINC

Physical and chemical properties of zinc, including its coordination flexibility, make it highly adaptable to meeting the needs of proteins and enzymes that carry diverse biological functions and are involved in the metabolism of proteins, nucleic acids, carbohydrates and lipids as well as in the control of gene transcription and other fundamental biological processes such as cell division, differentiation, development, immune phenomena and receptor activity. The advance in knowledge of zinc chemistry and biochemistry in the past two decades has been striking and reached a level that provides predictive capacity for both the physiology and pathology of zinc metabolism. The astoudingly large body of observations and an encyclopedic analysis of the data have been subject of numerous reviews [e.g.4,27,95,98], but surprisingly no attempt to discuss the roles of zinc in melanin-containing structures has been made.

4.1. Participation of Zn2+in melanogenesis

Catalytic function of Zn^{2+} in the synthesis of 5,6-dihydroxindole derivatives was noticed as early as 1950 [43] and included as a fact in the Raper-Mason scheme of melanogenesis. Observations of Prota and his associates have recently revived attention to the role of zinc in biosynthesis of melanins. They observed that various transition metals including Zn^{2+} affected markedly the chemical properties of melanin formed by the tyrosinase-catalyzed oxidation of L-dopa by increasing the incorporation of 5,6-dihydroxyindole-2-carboxylic acid into the pigment polymer [67,68]. Zn^{2+} can thus imitate function of dopachrome oxidoreductase. When acting together the inhibition of 5,6-dihydroxyindole-2-carboxylic acid decarboxylation was greater than that produced by Zn^{2+} or dopachrome oxidoreductase separately [50]. The suggestion that the presence of carboxylated indole units in natural melanins is due to the intervention in the melanogenesis of metal ions can be accepted. However, the role of Zn^{2+} namely in this respect appears to be uncertain because the free Zn^{2+} cation is damaging to biological systems and thus is associated with other molecules as Zn-ligand complex (see the section 3) resulting in a actual free Zn^{2+} ion concentration that is 10^{-3} - 10^{-6} that of the total zinc concentration [8,98]. Whether Zn^{2+}-ligand complexes can influence melanogenesis it has not been tested. Zn^{2+} ions were shown to inhibit the initial rate-limiting reaction of melanogenesis - tyrosine hydroxylation and thus to have a role in the regulation of melanogenesis [50].

4.2. Excretory function of melanosomes and pigment tissues

Melanin can participate in excretion of some substances under physiological conditions [81]. As hair melanosomes represent rich tissue reservoirs of zinc lost during removal of keratin structures, we tried to quantitate the Zn excretion via hair [12]. The daily Zn loss in man by this way varies around 20 μg which compared to the major Zn^{2+} portion excreted via pancreatic juice (10 mg/day) and to the output via urine (0.5mg/day) is low. However, if we add also Zn^{2+} loss by means of epidermal melanosomes, the value will increase.

4.3. Zinc and free radical processes

Since the 1970s it has been anticipated that an essential biochemical function of zinc is to serve as a natural antioxidant [20,99,100]. Two mechanisms of zinc action have been elucidated - the protection of sulfhydryl groups against oxidation and the inhibition of the production of reactive oxygen species catalyzed by some transition metals, especially by displaced iron [20,42,100].

On this basis it was predicted that relatively high concentration of zinc might be present in those tissues vulnerable to oxidation such as the hair, skin, eye and spermatozoa. When this was shown to be the case, Willson [100] proposed the following corrolaries: *1* -"in healthy cells, vital molecules are protected from the action of decompartmentalized iron by the presence of zinc"; *2* - "normal cells are designed in such a way that division is not initiated until the zinc concentration at critical sites within the cell is sufficient to protect them from decompartmentalized iron that might normally be present. Zinc thus plays protective and stimulatory role".

The frequent occurence of necroses in melanoma tissue [13] and the presence of H^2O^2 [24] make the metal driven free radical processes in pigmented tumours probable. Moreover, increased malondialdehyde levels found in the livers of B16 and S91 melanoma-bearing mice [13,71] suggest that the tumours alter host antioxidant defenses. Alteration of iron metabolism and increased levels of lipid peroxidation are characteristic of *substantia nigra* in Parkinson's disease [30] and the fact that also zinc levels in *substantia nigra* are markedly increased under these circumstances may indicate a physiological response to oxidative stress [29].

Melanin in melanosomes in pigment cells and tissues represents another source of free radical activity. The melanin polymer has long been known to exhibit stable free radical properties, because of semiquinones, which appear to have a protective action in cells probably by acting as a sink for diffusible free radical species [80]. Data derived from *in vitro* experiments have indicated that melanins can function as a scavenger of the superoxide anion radical and can protect cellular structures against photochemically induced lipid peroxidation also due to the absorption of light energy [35].

Zn^{2+} ions were shown to stabilize semiquinone anion radicals in melanin and to increase free radical activity in melanosomes [2,83]. Melanin polymerization is thought to occur by a free radical process in which semiquinones are formed by redox equilibration interactions between melanin precursors which as reactive species are strictly compartmentalized [13,80], and if leaked metabolically detoxified [13].

Evidence documenting that a number of catecholic melanin precursors, including cysteinyldopas and dihydroxyindoles, are photochemically unstable *in vitro* in the presence of biologically relevant ultraviolet radiation was presented by Koch and Chedekel [52]. Definitive evidence of occurrence of these reactions *in vivo* is currently unavailable, nevertheless these photochemical processes are expected to have a role in the pathogenesis of various pathological processes. The high level of zinc in epidermal and eye pigment cells may again indicate a physiological defense against the potential danger of oxidative stress.

4.4. Metal ion "buffering" by melanosomes - mobile pool of Zn^{2+}

Melanosomes have been proposed to represent a physiologically important "reservoir" for essential trace elements, a short term storage deposits, which by binding or releasing the metal ions may play a key role in the control of various processes, *e.g.* in the action of ionic pumps. Such mechanism is believed to be involved in the secretion of endolymphatic fluid in inner ear [60].

According to Pffeifer and Mailloux [69] melanin should be investigated as a storage bank for useful cations such as calcium, potassium, sodium and zinc. The binding of these ions would prevent a disruption in the body's osmotic balance. If the mineral balance was disrupted by dietary or physiological causes, the increased concentration of copper and lead with their greater affinity for melanin would lead to the displacement of more favourable cations - Zn^{2+} and Ca^{2+} which may have implication for hypertension and its therapy [69].

Scavenging role in the elimination of metals, when they reach too high levels in the cell, was ascribed to neuromelanin granules [101].

The complexity of zinc intercellular transport can be illustrated by earlier work of O'Rourke et al [64] demonstrating that zinc secreted by the ciliary body is made bioavailable and absorbed by the chorioretinal complex.

However interesting these theories sound, until zinc melanosomal binding sites and their binding parameters are clearly defined, we can hardly ponder upon the importance of these proposals. All we can say is that the melanosome pool of zinc is mobile as evidenced by the zinc release from eye melanosomes in the face of reduced amounts of bioavailable zinc, for example with a deficient diet [82].

4.5. Zinc and melanomas

Inhibition of tumour growth by dietary zinc deficiency appears to be a general effect irrespective of cell type, species or site of growth [49,89,96]. This may be mediated by the direct requirements for zinc for cellular proliferation as well as by indirect effects on immune function and the interaction with other trace elements.

As for melanoma, P51 mouse melanoma cells (derived from B16 melanoma) when grown in zinc-depleted media had longer doubling time and a decreased thymidine uptake [61]. On the contrary it was reported that the addition of zinc and iron tartrate complexes to Eagle's minimal essential medium was sufficient to support the proliferation of B16 melanoma cells in the absence of serum [54]. Altered organ distribution and survival of melanoma cells were observed in the Zn depleted dietary groups of P51 melanoma-bearing mice [61].

Zn^{2+} concentrations exceeding 10^{-4} mol/l are generally cytotoxic *in vitro* [14,15]. It is therefore not surprising that *in vitro* Zn^{2+} was shown to inhibit both the anchorage-dependent [14] and anchorage-independent growth [57] of Cloudman S91 melanoma . Attempts to suppress B16 and Cloudman S91 growth by zinc acetate administration in mice were unsuccessful because the necessary Zn^{2+} levels in vivo were difficult to reach [16]. Preincubation *in vitro* of cell suspensions with 10^{-4}

mol/l zinc acetate prior to injecting tumour cells inhibited melanoma development in mice [16]. 10^{-4} mol/l zinc sulphate was shown to decrease the *i.v.* but not *s.c.* transplantability of B16 melanoma [33].

Strong homeostatic control of zinc levels [4,27,95] prevents direct therapeutic use of zinc. The increased zinc uptake by melanomas might be rendered suitable for tumour localization with ^{69m}Zn [10] and for targetting tumour cells with chemotherapeutic agents since zinc may act as a carrier for pharmacologically active ligands [96].

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1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

Oxidation Chemistry: One electron oxidation of 5,6-dihydroxyindoline (DDI) or *N*-ethyl-DDI by azide radical and pulse radiolysis at pH 5 or 9 yields the benzosemiquinone radicals (pK_a 5.3) which disproportionate to yield a stable dopachrome-like product (*Alkazwini* et al.). In presence of Zn^{2+} at pH 5.0, benzosemiquinone radicals of DDI form a Zn ion complex of the *o*-semiquinone radical with a rate constant of 3.0×10^6 dm^3 mol^{-1} s^{-1}. This Zn ion complex decays by second order kinetics to yield a Zn^{2+}-quinone complex which has a lifetime of 3-4 ms. Autoxidation of DDI leads to formation of a dopachrome-like intermediate. From these studies, it is concluded that Zn2+ significantly influences the reactions involving the semiquinone radical of DDI and these alternative reaction pathways may help to clarify the initial biochemical stage of the free-radical pathway(s) leading to melanin formation.

Structure: During the reporting period, notable progress on structures of melanins has been achieved by application of MALDI mass spectrometry. *Napolitano* et al. analyzed model pigments prepared by enzymatic or chemical oxidation of DHI or DHICA. Marked differences in the nature of oligomer components were observed depending on the preparation conditions. No intact DHI oligomers were present in low m/z range between 500 and 1500, indicating a significant breakdown of the pigment backbone by peroxidative fission of indole units with concomitant decarboxylation and oxygenation reactions. Oxidation by means of tyrosinase in the presence of catalase reveals intact DHI oligomers up to hexamers. The molecular mass of DHICA melanin seems to be surprisingly low (< 1500). Also DHICA melanin is subject to peroxidative fission at the catechol site of the DHICA units, even if prepared under mild oxidation conditions. Polymerization intermediates (DP < 12), claimed to be melanochromes, were detected during the oxidative polymerization of DOPA, DHI, or DHICA (peroxidase/H_2O_2, mushroom tyrosinase/O_2, or autoxidation) (*Kroesche* and *Peter*). From time dependent spectra, it is concluded that melanins are formed by sequential coupling of monomers with concomitant oxygenation. The formation of melanin from 5,6- dihydroxytryptamine (5,6-DHT) (mushroom tyrosinase/O_2,) was studied by *Allegri* et al. Early reaction intermediates (30 min) corresponded to a series oligomers of 5,6-DHT (DP 2-8). Late products are the nonamer and secondary reaction products of lower oligomers, among them a compound claimed to be a macrocyclic structure. Studies by *Rosei* et al. on photoelectronic properties of melanins suggest that the pigments behave as an amorphous network of nanometer-sized conjugated clusters, where photogenerated electron-hole pairs undergo either geminate recombination or dissociation, depending on the photon energy.

Other papers deal with melanin formation in individuals exposed to high levels of radiation (*Pulatova* et al.), with the visual process in fish (*Brooks*), and with the oxidation of dihydroxybenzene in presence of ammonia (*Przegalinski* and *Matysik*).

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2. Biology of pigment cells and pigmentary disorders

(Comments by Dr M. Picardo)

Hair melanocytes are considered a reservoir for skin re-pigmentation during re-epithelization and in vitiligo subjects. These cells are difficult to identify because they are DOPA-negative. **Horikawa and co-workers** have found that the melanocytes of the outer root sheath of hair follicle can be detected by antibodies which recognise pre-melanosome related antigens but not using antibodies to tyrosinase either TRP-1 or TRP-2. Therefore melanocytes of the outer root sheath seem to contains the proteins of the early phase of melanogenesis but not those related to the later phase. Probably the stimuli able to induce the migration and re-pigmentation of the skin include the induction of enzymes and structural protein synthesis. In line with these findings, **Grichnik et al.** reported that KIT-reactive dendritic cells are identifiable in the basal layer of epidermis and most numerous in the follicular infundibula and the rete ridges. These cells are KIT+, BCL-2+ and TRP1- and can be differentiated from Merkel and Langerhans cells. The authors suggest that these cells can be precursors of melanocytes in human skin. Similarly, **Gilhar and co-workers**, reported that fetal melanocytes, which are normally DOPA negative, can be induced to synthesise melanin when transplanted into nude mice suggesting that these cells are potentially capable of synthesising melanin under condition different from those present in utero. On the contrary, the group of Thody presented interesting results on the reduction of C-KIT positive melanocytes in perilesional skin of vitiligo subjects. The authors speculate as to the possible biological meaning of these results and conclude that, whatever the cause, the alteration of c-Kit expression may well be responsible for the defect in the growth of vitiligo melanocytes. The results of these studies provide further improving in the biology of melanocytes indicating the possibility that "quiescent" melanocytes can exists and can be stimulated under peculiar situations.

Nakazawa and co-authors have focused on the role of PKC activation by phorbol esters on normal human melanocytes using a specific inhibitor of PKC. They demonstrate the critical involvement of PKC activation in TPA-dependent melanocyte growth stimulation, whereas morphology and adhesion to collagen IV seemed to be independent to the activation of PKC pathway. Phorbol esters are capable of increasing the adhesion of melanoma cells to extracellular matrix proteins. Studies on cell adhesion are performed to elucidate the mechanisms involved in tumor progression and metastasis. **Eguchi and Horikoshi**, have reported that treatment with phorbol esters increases the expression of integrin $\alpha 2\beta 1$, the receptor for laminin and collagen, and that the increased adhesion to type I collagen induced by TPA is mediated by the activation of calmodulin kinase and not via PKC.
Finally, **Wintzen and Gichrest** have presented an interesting review on the activities of proopiomelanocortin (POMC) and its derived on the skin including on melanocytes.

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Melanocyte cultures

(Comments by Dr N. Smit)

Both papers of Hunt et al. are of great importance for those who use cultured melanocytes as a model to study pigment metabolism. It is nicely shown that melanin content of cultured melanocytes differs from that in epidermis when the cells are maintained in (MCDB-153) culture medium. Our own recent studies have confirmed that this is also the case when Ham's F-10 medium is used. The increased levels of pheomelanin as found in the cultured melanocytes may strongly depend on the availability of cysteine and tyrosine in the culture medium. Also the differences in responsiveness of melanocytes from different skin types for MSH are highly interesting in the light of the recent observations by the same group that a variations in the MSH receptor gene are found in individuals with a light skin type (Valverde et al, Nature 11; 328-330, 1995). The transport mechanisms for amino acids into melanocytes and melanosomes as described for tyrosine by Gahl et al and Potterf et al may also be useful to understand pigment production in cells originating from different skin types. The skin equivalent model as described by Bessou et al may be valuable to study pigment production and the influences of UV-light. Melanin estimations should reveal whether in this system pigment production resembles the in vivo situation more closely than in melanocyte mono cultures so far. Methods for melanin estimations as described by Maeda and Fukuda and Schmidt et al may be helpful for such investigations.

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3. MSH, MCH, other hormones, differentiation

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4. Photobiology and photochemistry

(Comments by Dr M. d'Ischia)

It is uncommon that a literature search on a specific topic spanning over a few months encompasses so dense an array of outstanding contributions as does the present one. The mechanism of UV-induced skin-hyperpigmentation and the molecular bases of the pigmentation phenotypes and their tanning response figure prominently in the present survey. B. Gilchrest and her associates at Boston in a research paper (Proc Natl Acad Sci. U.S.A. 1996 Feb 6;93(3):1087-92)

and a reviewing article (Photochem Photobiol. 1996 Jan; 63(1):1-10) summarise the most significant achievements so far gained in their continuing efforts to dissect the complex links between UV irradiation and activation of pigment pathway. Experiments with Cloudman S91 murine melanoma cells as well as normal human melanocytes integrate and corroborate previous results by the same group pointing to DNA damage and/or its repair as crucial signals to activate melanogenesis. The list of stimulatory factors or mediators can be extended to include DAG and arachidonic acid, which are released from UV-irradiated membranes, and factors such as bFGF, NGF, MSH, which may act in concert to maintain and stimulate melanocyte activity.

A most relevant role, in this scenario, is played also by the MSH-MCIR receptor interaction. Valverde et al. (Nat Genet. 1995 Nov; 11(3):328-30) show that MC1R gene sequence variants in humans are generally associated with red hair and fair skin, as well as a low capacity to tan. The obvious conclusion is that the MC1R is a key control point affecting the pigmentation phenotype and the tanning response. Additional emphasis on the role of MSH and its receptors as mediators of the UV response of skin comes from a study by Chakraborty et al. (J. Invest Dermatol. 1995 Nov; 105(5):655-9) showing that UVB irradiation, exposure to MSH and dibutyryl cyclic adenosine monophosphate stimulate production of mRNAs for MSH receptors and proopiomelanocortin-derived peptides in mouse melanoma cells and transformed keratinocytes. It will be interesting to see how these end other exciting observations that are just behind the door will be integrated and assessed in the light of the current knowledge on the biochemical mechanisms affecting eumelanin vs. pheomelanin formation in epidermal melanocytes.

Mention is finally due to papers dealing with the EPR persistence measurement of light-induced melanin free radicals in whole skin (Photochem Photobiol. 1995 Sep; 62(3):557-60), and on the interaction of melanins with oxygen and carbon-centred radicals (Free Radic Biol Med. 1995 Dec; 19(6):735-40), which provide a useful model to address the protective role of melanin against peroxidation of the lipid components of melanosome membrane.

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Mechanisms of ultraviolet light-induced pigmentation. Photochem Photobiol. 63(1):1-10, 1996.

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Inhibitory effect of sodium 5,6-benzylidene ascorbate (SBA) on the elevation of melanin biosynthesis induced by ultraviolet-A (UV-A) light in cultured B-16 melanoma cells. Biol Pharm Bull. 18(8):1076-80, 1995.

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5. Neuromelanins

(Comments by Dr M. d'Ischia)

Although the neuromelanin containing human Substantia nigra has been the focus of intensive investigations because of its crucial role in disabling neurodegenerative disorders, there is at present no general consensus about its internal organisation. Mc Ritchie et al. (Neuroscience 1995 Sep; 68(2):539-51) provide a valuable contribution to this issue by quantitatively assessing the variability in the pattern of clusters of melanized neurons with serial section analysis and computer reconstruction. Based on the results of this study, the authors make a caveat about the widespread habit of evaluating topographical patterns of cell loss for diagnostic neuropathology using transverse sections of the Substantia nigra, since these are affected by marked variability. In contrast, horizontal sections of the human Substantia nigra exhibit much higher quantitative reliability, and as such should be preferably used for diagnostic neuropathology purposes.

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6. Genetics, molecular biology

(Comments by Dr F. Beerman)

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Commentary: The microphthalmia gene product, both in human (MITF) and mouse, is involved in melanocyte-specific transcription of tyrosinase and TRP-1 (ESPCR bulletin 22, 625-626, 1995). This paper analyses the regulatory elements in the human gene and the authors demonstrate preferential expression in melanocytes mediated by a melanocyte-specific promoter.

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Summary: A tyrosinase lacZ fusion gene, when introduced into transgenic mice, resulted in embryonic expression in presumptive pigment cells, but also in cell populations along the entire neural tube, with most prominent expression in developing brain.

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Proopiomelanocortin gene product regulation in keratinocytes. J Invest Dermatol 106(4):673-678, 1996.

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Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. Development 122(4):1207-1214, 1996.
Shortened abstract: Based on these data, we propose 4 distinct steps of embryonic melanocyte differentiation: (1) migration in the dermis, which requires both c-kit and endothelin 3; (2) a stage before epidermal entry that is resistant to anti-c-kit mAb; (3) cell proliferation after entering the epidermal layer, which requires c-kit and endothelin receptor B but not endothelin 3 and (4) integration into developing hair follicles, which renders melanoblasts resistant to anti-c-kit mAb, Thus, melanoblast differentiation proceeds by alternately repeating c-kit-dependent and c-kit-independent stages and c-kit functions as a survival factor for the proliferating melanoblasts.

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7. Tyrosinase, TRP1, TRP2 and other enzymes

(Comments by Prof. J.C. Garcia-Borron)

Two papers by Barbara Gichrest's laboratory discuss the mechanism of UV-induced pigmentation, and emphasize the activatory effect of DNA damage on melanogenesis (Gilchrest et al., 1996, Photochem. Photobiol. 63:1-10 and Eller et al., 1996, PNAS, 93:1087-1092). The observation that UV-induced DNA damage and photoproducts can directly stimulate melanogenesis is indeed beautiful in its simplicity. But the effects of UV light on melanocyte biology seem to be complex, in that they are not restricted to the control of melanin synthesis, but they extend to virtually all aspects of melanocyte biology. As pointed out by the authors, UV light might influence the rate of proliferation of melanocytes, as well as their shape, and their responsiveness to extracellular signals. The molecular basis of most of these effects are unclear and may involve interactions with chemical messengers derived from non melanocyte epidermal cells. In addition to presenting further evidence on the role of DNA damage in the control of pigmentation, the paper by Gilchrest's laboratory provide working hypotheses and a general framework useful to rationalize the complex effects of UV light on skin biology. Moreover, they emphasize the importance of the paracrine control of melanocyte biology. Indeed, the evidence pointing to a major role of chemical signals derived from skin cells in the control of melanocyte behaviour is accumulating at an increasing rate.

Solano et al. (Biochem. J., 313:447-453) describe the reconstitution of dopachrome tautomerase activity by addition of Zn (II) to apoenzyme preparations obtained by treatment of the purified enzyme with metal chelators. They suggest that Zn (II) might be the metal cofactor of DCT, which, owing to the properties of this metal cation, would account for its tautomerase activity, as opposed to the oxidizing activities of the cupper containing tyrosinases.
Reconstitution is a classical method to identify metal cofactors, and the technique is less prone to artifacts than other experimental approaches. However, recent work by Vincent Hearing's laboratory, presented in the last ESPCR Meeting, show poor binding of Zn (II) to DCT under "in vivo" conditions, thus casting doubts on the role of Zn (II) as the DCT cofactor. Further work will be necessary to reconcile these apparently contradictory observations on a topic particularly relevant to the mechanism of catalysis of DCT but also of the tyrosinases.

Nicklas and Sugumaran (Anal. Biochem., 230:248-253) present a somewhat improved version of a previously published method for "dopachrome isomerase (decarboxylating)" activity stain after electrophoretic separation. The method is in fact a minor modification of a previously published procedure, the main innovation being the use of periodate, instead of mushroom tyrosinase, for the "in situ" generation of the substrate, dopachrome. This method of dopachrome preparation has been used for years in many laboratories and presents, indeed, many advantages (including the cost!), although, as the authors rightly point out, excess periodate must be used with caution. It remains to be seen if the method is sensitive enough for the detection of dopachrome tautomerase activity in samples obtained from mammalian tissues, or if its use will be restricted to insects.

The paper by McLeod et al (J. Endocrinol. 146:439-447) presents further evidence that human melanocytes are able to respond to POMC-derived peptides with increased melanin pigmentation. Similar results have been reported by several other laboratories. The once-discussed ability of human melanocytes to respond to MSH appears therefore well established

nowadays. Conversely, the events coupling receptor activation and stimulation of melanogenesis remain obscure. McLeod and coworkers suggest a role for PKC in the signal transduction pathway. Englaro et al. (J. Biol. Chem. 270:24315-24320), using B16 mouse melanoma cells as a model, provide some evidence that the MAP kinase cascade might link increased cAMP levels and tyrosinase activation. However, an activation of the MAP kinase pathway following MSH treatment has not been detected in normal human melanocytes (Swope et al., 1995, Exp. Cell. Res. 217, 453-459). Whether these discrepancies reflect merely the use of different cell lines, or rather complex crosstalks events very sensitive to small differences in the experimental design remains to be seen. In any case, the study of signal transduction from the MSH receptor and its connections to other intracellular cascades, is a fascinating and rapidly moving field where exciting findings are expected in the near future.

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8. Melanoma and other pigmented tumours

(Comments by Dr N. Smit)

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Melanoma Experimental Therapy

Leonetti et al describe the possibilities to use antisense oligodeoxynucleotides (ODNs) targeted to the c-myc oncogene and their effects on melanoma cell proliferation. In the study by Jansen et al the use of Ha-ras targeted oligonucleotides was shown to employ antitumor effects which was not the direct result of specific inhibition of gene expression. The papers by Y. Ohta et al show that anti-oncogene ribozymes may also be useful for inhibition of melanoma growth.

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ANNOUNCEMENTS & RELATED ACTIVITIES

Also available in more details on address: **http://www.ulb.ac.be/medecine/loce/espcr.htm**

1996 **Skin Cancer and UV-Radiation - Bochum - Germany, 3 - 6 October**
Deadline for submission of abstracts : June 30, 1996
Preliminary scientific programme.
Contact: Dr Klaus Hoffmann
Dermatological Department Ruhr - University of Bochum
Gudrunstrasse 56
D - 44791 Bochum
PHONE : 49-228530890 - FAX: 49-228530986

1996 **EORTC BTDG Meeting**
King's College School of Medicine and Dentistry - London, 16 - 17 October

1996 **Fondation René Touraine - Journée scientifique 1996 - Paris, France, 25 October**
Le Mélanocyte, the Melanocyte
Contact: Fondation René Touraine - Hôpital St Louis - Service de Dermatologie
Avenue Claude Vellefaux
F- 75010 Paris
PHONE : 33-1-53722060 - FAX : 33-1-53722061

1996 **XVIth International Pigment Cell Conference - Anaheim, California, 29 October - 3 November**
Contact: MMC/UCI Center for Health Education
PO Box 1428, Long Beach
USA- CA 90801-1428
FAX: 310/933 2012 http://lenti.med.umn.edu/paspcr/ipcc.htm
Note from Prof. Martin G. Peter:
A limited number of travel stipends is available in form of a contribution to the cost of the rail or air ticket for students who wish to attend the meeting but cannot obtain support from institutional or personal funds or other sources. Applications should be sent together with a statement of the supervisor about non-availability of funds within six weeks after publication of this notice to the treasurer of ESPCR, indicating the reason for application and the approximate amount of money requested. The stipend will be paid after the meeting upon submission of the original spent travel documents. Applicants must be members of ESPCR.

1997 **4th World Conference on Melanoma - Sydney, Australia, 10 - 14 June**
Contact: The Melanoma Foundation
PO Box M123 - Camperdown
NSW 2050 Australia
FAX: 61 2/550 6316

1997 **VIIth PASPCR Annual Meeting RI - Providence, 15 - 18 June**
Contact: Dr. Walter C Quevedo, Jr.
Brown University, Division of Biology and Medicine
Providence, RI 02912
FAX: 401/863 1971

1997 **International Meeting "Pigmentary Disorders from a Global Perspective" - Bali, Indonesia, 22 - 24 June**
Contact: Bureau PAOG
Tafelbergweg 25
NL- 1105 BC Amstersdam
FAX: 31 20/696 3228

1997 **ESPCR Meeting: Bordeaux**
Contact: Dr Alain TAEIB
Hopital Pellegrin Enfants - Dermatologie
Place Amélie-Raba-Léon
F- 33076 BORDEAUX Cedex

Note in memory of Marcella Nazzaro-Porro

transmitted by Dr. Mauro Picardo

With the death in November 1995 of Marcella Nazzaro-Porro, the ESPCR has lost a cutaneous biologist of the highest standing in the field of melanin pigmentation. A pioneer member of our Society, from the date of the first European Workshop for Pigment Cell Research, she attended and participated at all the meetings, and her absence in the future will be sadly noted by members. She was born in Rome in 1926 and graduated as Doctor of Medicine in 1954. From 1955 to 1972 she worked as Associated Research Fellow first in the department of Dermatology at Rome University "La Sapienza" and then at the San Gallicano Dermatological Institute in Rome, directed at the time by her husband Prof. Paolo Nazzaro. In 1979 she was appointed Head of the laboratory of Research of Histopathology of the Skin at San Gallicano Dermatological Institute and she held this position until her official retirement in 1991.

Her works on skin pigmentation derived from studies on skin lipid composition and hypopigmentation occurring during the Pityriasis Versicolor. She and her colleague, Dr. Passi, showed that in culture of the fungus Pityriasis Versicolor. She and her colleague, Dr Passi, showed that in culture of the fungus Pityrosporum supplemented with unsaturated fatty acids, saturated dicarboxylic acids with chain lengths from C6 to C12 were generated. In vitro these diacid exerted an ascending gradient of competitive inhibition of tyrosinase and the thought arose that they may be of use in treatment of hyperpigmentary disorders in vivo. Attention was concentrated on one, Azelaic acid (C9 dicarboxylic acid) and its properties were compared with those of different phenolic substances alternative substrate of tyrosinase. From these earlier studies there developed over a period of 15 years a continuing tripartite international research collaboration involving teams at San Gallicano, the Dermatological Institute of the University of Turin and the Department of Anatomy of St Mary Hospital School London, aimed at further investigations on the biological properties and therapeutic potential of Azelaic acid. In these research, in addition to her individual contributions, Dr Nazzaro-Porro played a major inspirational and organisational role demonstrating her originality of thought, her sense of humour and her willingness to share her ideas with others and her ability to bridge the gaps between the different disciplines involved. The results are widely placed in literature and show Azelaic acid to be a remarkable molecule. The latter studies she conducted demonstrated that Azelaic acid is a scavenger of hydroxyl radicals and inhibits the HO mediated toxicity in cell cultures. The fact that Azelaic acid is generated by lipoperoxidation of delta-9- unsaturated fatty acids present in cell membranes and is a scavenger of free radicals led Dr Nazzaro to suggest that it can be regarded as a natural antioxidant belonging to cell defensive system.

Marcella Nazzaro-Porro had many interests outside of her work, especially in the fields of literature, music and the visual arts, in the pursuit of which she made many friends throughout the world. It is not only the scientific community which will mourn her untimely passing.

By Dr. Aidon S. Breathnach and Dr. Siro Passi

ESPCR Web Pages

Dear ESPCR member, Dear Colleague,

The President and Council members of the ESPCR decided to diffuse informations about the society throught the growing electronic networks worldwide. The utility of such a service is evident especially in providing freshly updated informations to you, in addition to the possibility of downloading usefull pages including coloured figures and photos. However, some of the informations will only be the privilige of ESPCR members and available through Keywords. The full text bulletin will be one of these.
Keywords will be regularly sent to you at your E-Mail address.

Below you will find the structure of the proposed Web pages,
you may also reach our site at: **http://www.ulb.ac.be/medecine/loce/espcr.htm**
Please note that many pages are still under construction.

Should you have any suggestions of any kind to help improving this service, please feel free to forward it to me on my Fax / E-Mail Address below.

G. Ghanem, ESPCR Bulletin Editor
Fax: 32-2- 534 95 50
E-Mail: gghanem@resulb.ulb.ac.be

Call for E-Mail addresses

Dear Colleague,

In order to improve our service to you, your E-Mail address is a valuable tool to diffuse useful informations very quickly.
PLEASE SEND a "Hello" to my E-Mail Address below and that's it. Thank you.

G. Ghanem, ESPCR Bulletin Editor
gghanem@resulb.ulb.ac.be

Meeting Report by Kazunori Urabe.

International Symposium on "Melanogenesis and Malignant Melanoma: Biochemistry, Cell Biology, Molecular Biology, Pathophysiology, Diagnosis and Treatment".
Fukuoka, Japan December 5-6, 1995

This symposium was held by Yoshiaki Hori, M.D., Professor and Chairman, Department of Dermatology, Faculty of Medicine, Kyushu University, to discuss new findings about melanogenesis, biochemistry of melanin, genetics of pigmented disorders, immunology of pigmented disorders and malignant melanoma, clinical features of malignant melanoma, prevention of malignant melanoma and treatment of malignant melanoma. Sixteen investigators were invited from abroad to report their new data on their fields and thirty Japanese ones were also invited to attend the meeting and sixteen of them presented their findings. The symposium was opened in the international conference room in the new modern building which was built for the international congresses a half year ago. Exact respected schedule by the speakers gave opportunity to everyone to hear and discuss what they were interested. There were five sessions in this symposium. The first session dealed with the biochemistry and molecular biology in benign and malignant pigment tissues. In this session, the relationship between genetic alterations in melanogenic proteins and the clinical pigment disorders were reported. And the biological characters of melanogenic cells affected by surrounding cells and the environmental stimulants were investigated. In the second session, immunological approaches for malignant melanoma were presented. Several specific antigens in melanoma were reported and the possibility for clinical application werediscussed. In the third session, the characteristic nature of melanoma cell were analyzed intensively. In the fourth session, some of new techniques for the diagnosis of malignant melanoma were presented. And the last session included the several new therapeutic methods; biochemotherapy, isolated limb perfusion, hyperthermia, and new products targeting melanoma. All papers weresummarized here. A full listing of abstract is available on request. However, papers reported in this meeting will be published in a book from Elsevier Science soon.

1) Biochemistry and Molecular Biology of Melanogenesis in Benign and Malignant Pigment Tissues
Matsunaga J from Akita University of Medicine reported the mutations in the tyrosinase gene causing tyrosinase-negative oculocutaneous albinism in Japanese. In these nine patients, mutations of the tyrosinase gene were found at codon 77, codon 278, codon 310 and codon 431 of the gene. He suggested that in these mutations, codon 77 and codon 310 might be major mutation sites in Japanese patients. King RA from University of Minnesota demonstrated eleven mutations (9 unique and 2 previous reported) of the P gene which is responsible for tyrosinase positive oculocutaneous albinism. Hermansky-Pudlak syndrome was also intensively studied by his group, which is a type of albinism associated with storage-pool deficient platelets and with the production of ceroid. Linkage analysis of the family revealed that the gene was located on chromosome 10q. Further studies were going on to clone the gene. I presented the data about the regulation of microphthalmia (mi) gene of which mutations induced white hair, microphthalmia and deafness in mice. The expression of mi gene was not affected by MSH stimulation. Using neural crest cells, Kubota Y from St. Marianna University in Kawasaki suggested that the stem cell factor might play a role in the development of c-kit positive cells from neural crest and that there was a critical time (Days 0-5) when the stem cell factor induce c-kit positive cells and that other factors such as cholera toxin might be necessary to induce further differentiation of the cells to melanocytes. Meyskens FL from University of California focused on the transcription factors of human melanocyte in response to ultraviolet radiation. His group demonstrated

the high dose of 500 mJ of ultraviolet induced AP-1 and down regulated NFkb and that normal human melanocytes required both the PKC and PKA signaling pathways for UVB induction of AP-1 and NFkb. To elucidate the paracrine linkage of cytokines between human keratinocytes or fibroblasts and melanocytes for biological mechanisms of involved in cutaneous melanosis, Imokawa G from Kao Corporation characterized keratinocyte- and fibroblast-derived factors responsible for proliferation of melanocytes. They showed that IL-1a and endothelin(ET)-1, and GM-SCF were predominantly produced by keratinocytes in response to UVB and UVA irradiation, respectively, whereas stem cell factor (SCF) was the major cytokines produced by fibroblasts, and that these factors were synergistic of additive stimulatory effects on DNA synthesis of cultured melanocytes. Prota G from University of Naples suggested that melanin-related metabolites might play a critical role of the well being of both melanocytes and the surrounding cells. He showed that DHI was capable of inhibiting lipoxygenase-induced oxidation of arachidonic acid, a primary event in inflammatory reactions, and that DHI and DHICA were also endowed of excellent antioxidant properties and were capable of scavenging oxygen radicals. Another study was also presented that DHICA was a potent enhancer of nitric oxide production by LPS stimulated murine macrophages. To characterize melanins in mouse and human hair of various colors, Ito S from Fujita Health University School of Health Science presented new findings in their methods; 1) solubilization of hair melanins in Soluene-350 was a convenient method to estimate the total amount of melanin. 2) chemical differences among melanins produced in the brown, slaty, and other colored mice could be elucidated by HPLC and spectrophotometric methods. 3) eumelanin to pheomelanin ratio could be estimated from the slope of absorption spectrum of melanin dissolved in Soluene-350. Demonstrating the effects of prostanoids, leukotrienes, lymphokines, cytokines, melanotropins, endothelins and other factors on melanocyte on various conditions, Nordlund JJ from University of Cincinnati concluded that the concepts about the melanocyte and its receptors must be updated to show the multiplicity of receptors that made the cell responsible to many cytokines, lymphokines and chemical mediators of inflammation, and that the melanocyte itself had the same capabilities for forming these various factors as other cells, and that the formation of these factors by the melanocytes suggested that melanocytes had both autocrine and paracrine roles for all epidermal cells including Langerhans cells. Kikuchi K from University of Tokyo examined the ET receptor subtypes involved in mitogenic signaling in human primary and metastatic melanoma, and suggested that the mitogenic effects of ET in human primary melanoma were mainly dedicated through ETB receptors, and that down-regulation of ETB receptors caused the decreased growth response of ET-1 in metastatic melanoma cells.

2) Immunological Approaches for Malignant Melanoma Itoh K from Kurume University showed that the MAGE-1 and -4 proteins which were tumor-rejection antigens recognized by cytotoxic T lymphocytes, were expressed in many different cancers including melanoma, and in spermatogonia and primary spermatocytes. And he suggested that MAGE proteins might be appropriate target molecules for specific immunotherapy of cancer. Ferrone S from New York Medical College Valhalla presented the data of immunotherapy with the anti-idiotypic (anti-id) antibodies. He discussed the rationale underlying the selection of anti-id mAb as immunogens and of human high molecular weight-melanoma associated antigen as a target, and described the immunogenic and structural characteristics of the anti-id mAb MK2-23. He also presented the results of active specific immunotherapy with mAb MK2-23 in 50 patients with advanced melanoma. To reduce the immunogenicity of anti-id mAb MK2-23, Matsumoto K from Shinshu University School of Medicine evaluated the in vitro reactivity and immunogenicity of F(ab')2 fragments of mAb MK2-23 and of its chimeric form, and suggested that these might be useful immunogens to implement active specific immunotherapy in the patients with malignant melanoma. Hayashibe K from Kobe University School of Medicine analyzed a human melanoma-associated antigen D-1 which was identified by his group. They demonstrated that D-1 antigens were specifically expressed in melanoma cells and that HLA A33 might be associated with high expression group of D-1 antigen in patient with malignant melanoma. Taniguchi M from Chiba University investigated TCR repertoire in tumor-infiltrating lymphocytes in metastatic melanomas. He

reported that only two TCR Vas, such as Va3+ and Va4+ TCRs, dominated and comprised about 75% of total TIL TCR Va repertoire, and that the majority of these two TCRs were extremely homogenous. And he also demonstrated that depletion of Va3 T cells by anti-Va3 resulted in protection against melanoma lung metastasis. Hearing VJ from National Institute of Health in Bethesda showed that many spontaneous autoimmune responses against melanocytes, including those directed against melanoma cells, reacted with epitopes derived from melanosomal proteins. He demonstrated that TRP2 was the most generally expressed of those potential antigens and thus might be the most appropriate target for vaccine strategies.

3) Biochemical Analysis on the Expression of Specific Proteins in Melanoma Horikoshi T from Sapporo Medical University reported the serum 5-S-cysteinyldopa (5-S-CD) level reflects melanoma progression more sensitively than urinary 5-S-CD, serum or urinary DHICA, and suggested that serum 5-S-CD might be the best biochemical marker for the detection of progression of melanotic melanoma. Melanin-producing cells are subject to a high risk of oxidative stress, particularly due to the presence of melanogenic machinery continuously producing o-quinones, the precursors of polymer melanin. Pavel S from Leiden University Hospital suggested that 0-methylation was one of the protective means decreasing redox cycling potential of melanin precursors and speeding up their transmembrane transport, and that L-cysteine and glutathione not only participated in the redox reactions but also controlled the quality and quantity of produced melanin. Taniguchi S from Shinshu University of Medicine found and cloned a variant actin (bm actin) which was responsible for the decrease of metastatic ability of mouse melanoma cell. When the bm actin cDNA expression vector was transfected into a highly metastatic cell lines, he observed the inhibitory effects on the cell motility, invasion, and metastatic ability depending on the expression of the exogenously transferred bm actin. He suggested that bm actin inhibits the dynamic conversion between the monomeric and polymerized form of actin, leading to both a decrease in cell motility and consequently the suppression of invasiveness and metastasis. Kageshita T from Kumamoto University School of Medicine examined 58 primary and 35 metastatic melanoma and 22 pigmented nevi using anti-vitronectin receptor (VN-R) chain antibody, and found that VN-R avb3 chains were expressed in 47, 34 and one samples, respectively. He suggested that the expression of VN-R avb3 chain in melanocytic tumors was correlated with development of deep invasion and metastatic process. Tsuchida T from Saitama Medical School demonstrated that among gangliosides, GM3 and GD3 was predominant in congenital pigmented nevi and primary malignant melanoma, respectively. And he also reported that moderate amounts of sulfatide were detected in congenital pigmented nevi and primary malignant melanomas, but not in metastatic melanomas.

4) Diagnosis of Malignant Melanoma Malignant melanoma has a variable prognosis determined by several specific histologic features. As the histopathologic variables, Mihm MC from Albany Medical College listed these parameters;
1. tumor thickness, 2. level invasion, 3. mitotic rate (per millimeter square), 4. tumor infiltrating lymphocytes, 5. regression, 6. ulceration, 7. predominant cell type morphology, 8. microscopic satellites, 9. vascular invasion, 10. nodular growth (vertical growth phase). Of these parameters, he emphasized tumor infiltrating lymphocytes and discussed the predominant cell morphology, its cell biologic implications as well as its molecular characteristics and how they related to prognosis. Hara H from Nihon University School presented several methods for the detection of melanogenic and proliferative activities on malignant blue nevus; formaldehyde-induced fluorescence from formalin-fixed, paraffin-embedded materials, HPLC analysis of 5-S-cysteinyldopa from frozen specimen, argyophilic nucleolar organizer regions (AgNORs) and cytofluorometry. He concluded that the cells on malignant blue nevus showed a melanogenic activity and that cytofluorometric analysis could be regarded as a useful parameter for the determination of proliferative activity. Investigating the videomicroscopic features of 500 melanocytic nevi on the soles, Akasu R from Yamanashi Medical University indicated that the surface profile of benign melanocytic nevi was classified into 5 types,

and that malignant melanoma in situ and acral lentiginous melanoma were exclusively compartmentalized in the miscellaneous type. And she suggested that epiluminescence microscopy might be a useful method for discrimination of plantar benign and malignant melanocytic lesions and might be useful for long-term follow-up of the melanocytic lesions. Sober AJ from Harvard Medical School presented several new techniques in the early diagnosis of melanoma; an epiluminescence microscopy, a computerized image analysis and a confocal laser microscopy. Confocal laser microscopy utilizes an argon, krypton, or Ti:sapphire laser into a confocal scanning microscope producing high resolution images. His group was investigating the use of this technique in the imaging of pigmented lesions and assessing the clinicopathologic correlation. McCarthy WH from Royal Prince Alfred Hospital in Sydney used lymphoscintigraphy to mark the surface location of the sentinel lymph node in each node field and to measure the depth of the node, and demonstrated that lymphoscintinography was accurate in 97% of patients in identifying potential sites of micrometastses of sentinel nodes.

5) Treatment of Malignant Melanoma Jimbow K from University of Alberta in Canada investigated the anti-melanoma effect of the phenolic thioether amines for the development of a new targeted chemotherapy of radiochemotherapy for malignant melanoma. He evaluated the specificity and improved effectiveness of N-propionyl cysteaminylphenol (NPrCAP) and its prodrug dipropionyl CAP (DPrCAP) over N-acetyl CAP (NAcCAP) and its prodrug of diacetyl form (DPrCAP) as antimelanoma agents, and the mechanism of drug action of NAcCAP and NPrCAP focusing on the production of quinone/semiquinone radicals mediated through interaction with tyrosinase and other oxidases. Cascinelli N from National Cancer Institute in Milano discussed three specific and actual subjects of high interest: adoption of a new technique for an earlier detection of nodal metastases, gene therapy of metastatic disease and interferon in the adjuvant therapy of patients with nodal metastases. Riley PA from University College London Medical School tested sixteen phenolic compounds for their ability to act as substrates for tyrosinase and their cytotoxic potentials. He revealed that protection of the hydroxy group by acetylation or glycosylation prevented in vitro oxidation by tyrosinase, and that both acetate and succinate esters were cytotoxic, and that protection of the phenolic OH group by sugars abolished in vitro cytotoxicity. Isolated limb perfusion (ILP) with melphalan produces a 50% complete remission rate in melanoma and a 6% in sarcoma of the limbs. In order to improve these results, Lejeune FJ from Universitaire Vandois in Switzerland added rTNFa to melphalan and rIFN-g in ILP for in-transit melanoma metastases, irresectable soft-tissue sarcomas and carcinomas of the limbs. A 90% complete response rate was obtained for melanoma, a 36.4% complete response rate for sarcoma and a 57% complete response rate for squamous cell carcinoma. Thioureylenes such as 2-thiouracil are known to be selectively accumulated in nascent melanin. On animal experiments, Larsson BS from Uppsala University in Sweden showed that the thioureylenes might be used as vehicles of radionuclides for melanoma scanning or treatment, and of boron-10 for neutron capture therapy. He also indicated that radioiodinated 2-thiouracil might be useful in the diagnosis of disseminated malignant melanoma. Hyperthermic isolated limb perfusion with the infusion of chemotherapeutic agents and/or cytokines has been proven to be definitely effective for the treatment of malignant melanoma at least locoregionally. Nakayama J from Kyushu University studied the mechanism of cytotoxic effects of hyperthermia on melanoma cells, and indicated that hyperthermia caused activation of immune system of the host, probably through the upregulation of ICAM-1 expression in melanoma cell and/or immune competent cell of the host.

INTERPIG DataBase (by Vincent Hearing)

The INTERPIG database is on the InterNet! You can now access the InterPig DataBase at the following address: **http://lenti.med.umn.edu/paspcr/interpig.html.**
Please note that as of this time, I estimate that less than 5% of the various IFPCS members have contributed entries. Think of how useful and complete this list would be if everyone took the time to supply their own information. Please take a moment to fill out the database data entry form and send it back to Dr. Hearing.

XVIth International Pigment Cell Conference Official Program

Tuesday, October 29, 1996

3:00 - 7:00 pm	Pre-registration/View Exhibits
7:00 - 10:00 pm	Welcome Reception: Fashion Show: "Safe and Sexy in the Sun"

Wednesday, October 30, 1996 Conference Attendees

7:00 - 8:00 am	Registration/Continental Breakfast/View Exhibits
8:00 - 8:05 am	Welcome: Chairman, Frank L. Meyskens, Jr.
	Introduction: Laurel Wilkening, Chancellor, Univ of California, Irvine
8:05 - 8:35 am	Special Lecture, R. Sherwood Rowland, Nobel Laureate, 1995, Chemistry
	"Ozone Depletion, Ultraviolet Light, and the Pigment Cell"

Symposium I: Economic and Societal Implications of Melanin and Melanogenesis

8:35 - 9:00 am	Keynote Speaker
9:00 - 10:30 am	Invited and Competitive Abstract Speakers
10:30 - 11:00 am	Break
11:00 - 12:30 pm	Workshop A: "Extracutaneous Melanin, Melanocytes and Melanogenesis"
	Workshop B: "Dynamics of Invertebrate Pigment Cells"

Posters and Discussion #1 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)

12:30 - 2:00 pm	Lunch on your own

Symposium II: Molecular Biology of Pigment Cells

2:00 - 2:30 pm	Keynote Speaker
2:30 - 4:00 pm	Invited and Competitive Abstract Speakers
4:00 - 4:05 pm	IFPCS InterPig Database on the WorldWideWeb: Vincent Hearing
4:05 - 4:15 pm	Break
4:15 - 6:15 pm	Workshop C: "Regulating Mechanisms of Melanocyte Proliferation"
4:15 - 6:00 pm	Posters and Discussion #2 TBN* (4:15 - 5:30 Viewing; 5:30 - 6:00 Discussion)
5:30 - 7:00 pm	Workshop D: "Biophysics and Chemistry of Melanin"
	Workshop E: "Vitiligo"
6:15 pm	Adjourn - Free evening

Accompanying Guests

9:00 - 11:00 am	Welcome/Introduction: Buffet Breakfast
10:00 - 11:00 am	Orientation
11:00 - 6:00 pm	Group Activity - Huntington Library

Thursday, October 31, 1996

7:00 - 8:00 am	Continental Breakfast/View Exhibits
8:00 - 8:30 am	Seiji Lectureship: Introduction: Giuseppe Prota, President, IFPCS

Symposium III: Melanoma Research: Basic and Applied

8:30 - 9:00 am	Keynote Speaker
9:00 - 10:30 am	Invited and Competitive Abstract Speakers
10:30 - 11:00 am	Break
11:00 - 12:30 pm	Workshop F: "Control of Melanogenesis"
12:30 - 1:30 pm	Simultaneous Business Meetings of Regional Societies
12:30 - 2:00 pm	Lunch on your own

Symposium IV: Photobiology of Melanocytes: Etiology and Prevention

2:00 - 2:30 pm	Keynote Speaker
2:30 - 4:00 pm	Invited and Competitive Abstract Speakers
4:00 - 7:00 pm	Workshop G: The "Blues" Symposium
4:00 - 7:00 pm	Poster Viewing

Adjourn - Free evening

Friday, November 1, 1996

7:00 - 8:00 am	Continental Breakfast/View Exhibits
8:00 - 8:30 am	Introduction: Sally Frost-Mason, President, PASPCR
	Gelb Lectureship: Seth Orlow

Symposium V: Melanogenesis and Pigmentary Disorders

8:30 - 9:00 am	Keynote Speaker
9:00 - 10:30 am	Invited and Competitive Abstract Speakers
10:30 - 11:00 am	Break
11:00 - 12:30 pm	Workshop H: "Biology and Biochemistry of Melanosomes"
11:00 - 12:30 pm	Posters and Discussion #3 (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 1:30 pm	IFPCS Business Meeting
1:30 pm	Adjourn Scientific Session
1:30 - 6:30 pm	Break
6:30 - 7:30 pm	Reception
7:30 -	midnight Banquet, Awards and Dancing

Saturday, November 2, 1996

7:00 - 8:00 am	Continental Breakfast/View Exhibits
8:00 - 8:30 am	Presidential Address: Giuseppe Prota, President IFPCS

Symposium VI: Comparative Developmental Biology of Pigment Cells

8:30 - 9:00 am	Keynote Speaker
9:00 - 10:30 am	Invited and Competitive Abstract Speakers
10:30 - 11:00 am	Break
11:00 - 12:30 pm	Workshop I: "Genetic Aspects of Albinism"
	Workshop J: "Melanocytic Nevi: Clinical and Laboratory Investigations"
11:00 - 12:30 pm	Posters and Discussion #4 (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 2:00 pm	Lunch on your own
2:00 - 4:00 pm	Educational Forum: "Living with the Sun"
4:00 - 6:00 pm	Family Farewell Reception and Wine Tasting

Sunday, November 3, 1996

8:00 - 5:00 pm

1. Satellite Meeting (all day): Classification of Cutaneous Melanoma: Alistair Cochran
2. Satellite Meeting (3 hours): Safety of Sunscreens and Tanning Parlors: J.P. Cesarini, et al. (Morning)
3. Satellite Meeting (3 hours): Ocular Melanin: Giuseppe Prota (Afternoon)

Workshops and poster and poster discussion sessions will be simultaneous.
The poster sessions and discussions will feature areas that do not overlap with the workshop. The chairs of these sessions will be selected from submitted competitive abstracts and the Chairman in turn will organize this session with help from the Organizing Committee.