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

Discussion

Alkylating melanotropin fragment analogs

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All knowledge acquired by investigations on biological activities, structure-activity relationships and mechanism of action of peptide hormones mainly serves therapeutic purposes. In endocrine disorders the use of good agonists or antagonists is necessary to mention only the clinical application of insulin, ACTH, TRH, LH-RH and their analogs, while in other cases the peptide hormones are only carriers of drugs, taking advantage of their specific receptor recognizing ability.

Whichever may we wish to use peptide hormones in therapeutics, the main requirement is that the hormone or its analog should possess only one single biological activity so that it should only bind to the cells of the targeted organ. This aspect has come all the more into the foreground since in the course of far-reaching investigations on peptide hormones it turned out that most of them exert various kinds of physiological activities meaning that they may have various receptors on different cells.

This is the case with the α-melanocyte stimulating hormone (α-MSH) too, affecting beside its main biological activity (pigment dispersion, skin darkening), the nervous and immune system as well (1). These biological activities constitute the basis of the biomedical application of the α-MSH. In the course of the study of its structure and melanocyte stimulating activity relationships superpotent agonists (2) and antagonists (3) were developed as promising candidates for the treatment of pigment disorders, while among its analogs acting on CNS there are some which are able to improve memory (4). In the therapy of melanomas containing α-MSH receptor positive cells a good cytostatic effect may be expected by using melanotropin antagonists similarly as LH-RH antagonists used in the treatment of prostate cancer (5), or by the application of conjugates containing the hormone and a known cytotoxic agent. Melanotropin antagonits are already available as mentioned above, although to our best knowledge their cytostatic activity has not yet been investigated. As carrier molecule, ß-MSH was used, and its conjugate with Daunomycin (6) showed a selective toxicity in vitro on Cloudman S91 mouse melanoma cells, but there has been no report on in vivo results.

In our conception there is no need for the whole hormone molecule for targeting, since it has been known for a long time that smaller fragments of α-MSH also exert biological activity even if their effect is many orders of magnitude behind that of the parent hormone.

The central His-Phe-Arg-Trp sequence can be regarded as the main active center (7), although Eberle et al (8) reported the presence of a second active center the C-terminal Lys-Pro-Val-NH_2 sequence, and Medzihradszky and Medzihradszky-Schweiger (9) found other smaller fragments to be active as well in the frog skin bioassay (10). As these investigations were performed in different laboratories and occasionally even on skins of different Rana species, Hruby and coworkers made systematic investigations with peptides uniformly acetylated on their N-terminus and amidated on their C-terminus and their results in the frog skin assay only pointed to the presence of one single centrally located active center. However, their investigations do not preclude the possibility that other melanotropin fragments may be active in another species, or on other than pigment cells, like the Trp-Gly-Lys-Pro-Val-NH_2 C-terminal pentapeptide which is active in the tyrosinase assay performed on Cloudman S-90 melanoma cells (12).

Thus, to obtain targeting conjugates, we chose both central and C-terminal fragments of the α-MSH, like the 5-10 hexapeptide, the 11-13 tripeptide, the 10-13 tetrapeptide, and the 9-13 pentapeptide. The most obvious site for the modification of the peptides with the drug seemed to be the N-terminal part, since the native hormone possesses no free amino terminus, and the biological activity of the smaller fragments also changes favourably with acetylation.

As a first approach (13) we substituted the amino terminus of the peptides by the N-(2-chloroethyl)-N-nitrosocarbamoyl (QNO) group, which is the functional group of such effective antitumor agents as BCNU, CCNU and Chlorozotocin. Since in the case of QNO-Glu-His-Phe-Arg-Trp-Gly-OMe we always observed some denitrosation attributable to the presence of the γ-carboxyl group of the glutamic acid, we synthesized an analog of this central sequence where glutamic acid was substituted by glycine and L-phenylalanine by D-phenylalanine to prevent rapid enzymatic degradation of the peptide moiety in the circulation. In this way we got a superactive hexapeptide which was only by one order of magnitude less potent in the frog skin assay than the native tridecapeptide hormone (14).

In the other group of conjugates (15) for peptide carriers we chose about the same melanotropin fragments as before and as alkylating agent the phenylalanine mustard (PAM). As compared with the QNO group, we thought that the following characteristics of PAM would be more favourable for our purposes: i) being an amino acid derivative, it can be incorporated into each position of the carrier peptide, ii) the mustard group is more stable than the QNO one and iii) the reaction of the mustard group is unambiguously alkylation, while the chloroethylnitrosourea conjugates have a carbamoylating activity beside the alkylating activity as well.

After synthesizing these melanotropin fragment derivatives the question arose, whether the modified peptides preserved their receptor recognizing ability. Since MSH receptors have been cloned only recently (16), the receptor recognizing ability of the peptide conjugates could only have been checked indirectly, by measuring their biological activity (14). As it can be seen in Table I., the melanocyte stimulating activity of the acetyl and QNO-peptides does not differ significantly, the decrease in the latter case not exceeding one order of magnitude.

The melanotropin fragments containing L- or D-PAM have approximately the

same melanin dispersing activity as the parent peptides. In Table I. we have listed only those derivatives which contain the L isomer, Melphalan (Mel), and which were further examined. Since both types of congeners possess biological activity and presumably receptor recognizing ability too, at least in the frog skin bioassay, the basic condition of targeting seems to be fulfilled.

It is well known that some of the α-MSH analogs and fragments possess prolonged biological activity (17), which means that the skins darkened by the agonist do not lighten to the original colour after washing as the native hormone does.

Table I. The melanocyte stimulating activity of α-MSH fragments and their derivatives

No.	Peptides	Activity U/mmol	
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	$4x10^{10}$	
	Ac-Lys-Pro-Val-NH_2	$3x10^{4}$	
I	QNO-Lys-Pro-Val-NH_2	$6x10^{3}$	
	Ac-Gly-Lys-Pro-Val-NH_2	$5x10^{4}$	
II	QNO-Gly-Lys-Pro-Val-NH_2	$5x10^{3}$	
	Ac-Trp-Gly-Lys-Pro-Val-NH_2	$6x10^{5}$	
III	QNO-Trp-Gly-Lys-Pro-Val-NH_2	$4x10^{4}$	+
	Ac-Gly-His-D-Phe-Arg-Trp-Gly-OMe	$1x10^{9}$	
IV	QNO-Gly-His-D-Phe-Arg-Trp-Gly-OMe	$4x10^{8}$	+
	H-Lys-Pro-Val-NH_2	$1x10^{4}$	
V	Mel-Lys-Pro-Val-NH_2	$1x10^{4}$	+
	H-Trp-Gly-Lys-Pro-Val-NH_2	$7x10^{4}$	+
VI	Mel-Trp-Gly-Lys-Pro-Val-NH_2	$2x10^{4}$	+
VII	Phe-Glu-His-Phe-Arg-Trp-Gly-OMe	$2x10^{6}$	+
VIII	Mel-Glu-His-Phe-Arg-Trp-Gly-OMe	$3x10^{6}$	+
IX	Nle-Glu-His-Mel-Arg-Trp-Gly-OMe	$2x10^{6}$	+

+ Indicates prolonged biological activity.

Interestingly not all of the alkylating derivatives cause prolongation of the darkening effect, while some of the normal (unsubstituted) peptides do. For closer investigation of this phenomenon we have performed inhibitory experiments with the peptides marked with +, and the results are presented in Fig. 1. It can be seen that only after the treatment with Phe-Glu-His-Phe-Arg-Trp-Gly-OMe and Mel-Lys-Pro-Val-NH_2 could the full darkening effect of the α-MSH be achieved, while the other derivatives exert inhibitory effect. Accordingly, when Mel replaces methionine, arginine or phenylalanine in the original sequence and in the case of the QNO derivative of the central melanotropin fragment a prolonged action and inhibition occur. Although not proved yet, we think that in these cases a covalent bond is formed between the alkylating group and an appropriate nucleophile on the receptor site to which the Met-Glu-His-Phe-Arg sequence fits. The reason for the long lasting effect of the other peptides, including normal peptides mentioned in the literature without any chemically reactive group, is still unexplained.

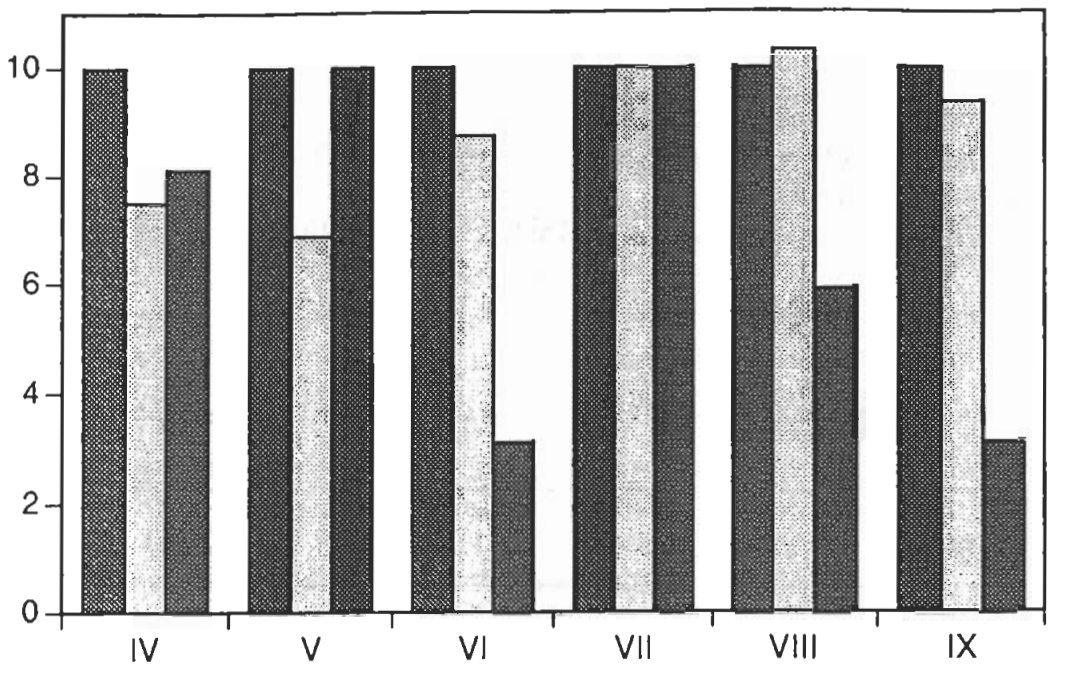

Fig.1 The inhibitory effect of alkylating α-MSH fragments in the frog skin bioassay measured in the following steps:
darkening by α-MSH; washing, darkening by the Mel-fragment; washing, darkening again by α -MSH. For detailed data see ref 15.

The antitumor activity of the alkylating melanotropin fragments has been tested in several in vivo and in vitro systems. The QNO-peptides generally cause a higher increase in the life span of L1210 leukemia bearing mice than the parent antitumor drug BCNU they were nearly equally active on human melanoma xenografts in mice and no effect was observed on human colon xenografts in mice (18). It is interesting that the great differences in the melanocyte stimulating activities (4-5 orders of magnitudes) of the conjugates are not reflected in the melanoma growth inhibitions. The members of the Melphalyl peptide group were effective in the increasing of the life span of L1210 leukemia bearing mice similarly as Melphalan, the only exception was VI with a significant higher effect, and they were roughly equally potent on human melanoma xenografts (19).

The failing of the desired high effect in the case of both types of peptide conjugates on melanoma xenografts does not really contradict the receptor mediated action, the reason for it may be simply the low number of MSH receptors available on the cell surface and, consequently, a relatively low concentration inside of the cell. Moreover it is also known that not all types of human melanoma cells contain α-MSH receptors (21). Therefore we have chosen several Mel conjugates for further investigations which were carried out by Ghanem and his coworkers at the Free University of Brussels (22). The receptor binding ability of the Mel containing derivatives V, VI, VIII and IX was checked on an α-MSH receptor positive human melanoma cell line against ^{125}J-(Nle^4, D-Phe^7) α-MSH. Only the central hormone fragment derivatives

VIII and IX are competing with the labelled hormone similarly to the natural α-MSH$_{4-10}$ sequence, and when the cells were preincubated with them, the α-MSH binding was significantly inhibited. It is worth recalling that VIII and IX exerted inhibitory effect on the biological activity in the frog skin bioassay, too (Fig. 1).

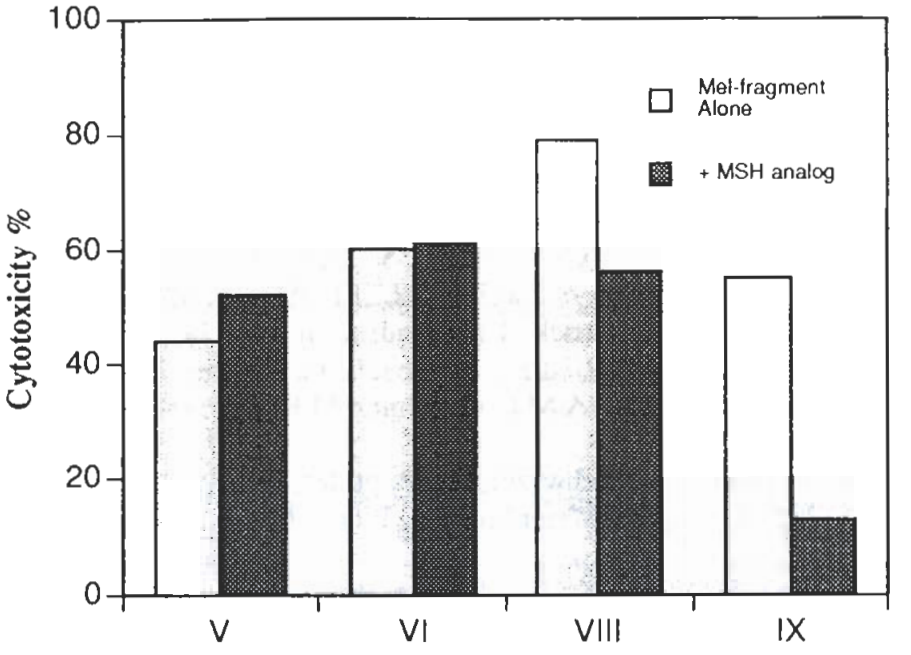

Fig 2. The inhibition of the cytotoxic effect on melanoma cells of Mel containing α-MSH fragments by the superagonist (Nle^4, D-Phe^7) α-MSH. For detailed data see ref. 22.

The cytotoxic effect of V, VI, VIII and IX was tested by the ^{3}HTdR uptake assay on human cell lines. The comparison of the drug concentrations inhibiting 50% of cell growth indicates a selective cytotoxic effect on the melanoma cells in the case of VIII and IX which are about six times more toxic on melanoma than on fibroblast cells, while V and VI are equally toxic on both cell lines. When melanoma cells were preincubated with a known α-MSH superagonist (Nle^4, D-Phe^7) α-MSH (23) no change in the cytotoxic effect of the C-terminal sequence analogs V and VI occurred, while in the case of the receptor specific central fragment analogs a significant decrease in the activity was observed (Fig. 2), indicating the receptor mediated cytotoxic effect of these congeners.

Although not strictly connected with targeting, an advantage of the conjugates should be mentioned, namely their diminished mutagenic effect on normal human lymphocytes as compared to that of Melphalan (20).

Summing up, the synthesis of alkylating analogs of α-melanotropin fragments, and the investigation of their biological activity may offer answers to some important questions. A number of analogs prove to be irreversible inhibitors, or tightly bound ligands with prolonged biological action. Some conjugates show lower toxicity and better therapeutic indices than the alkylating compound itself. These observations point to the possibility of developing alkylating peptide derivatives advantageously applicable for therapeutic purposes.

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CURRENT LITERATURE

We acknowledge the valuable assistance of
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1. Melanins and other pigments chemistry

- Aime S, Fasano M, Terreno E, Groombridge CJ.
NMR studies of melanins: characterization of a soluble melanin free acid from Sepia ink. Pigment Cell Res 4:216-221, 1991.
Abstract : This paper deals with the nuclear magnetic resonance characterization of a soluble derivative (melanin free acid) of Sepia melanin obtained by a peroxidative treatment of the parent (insoluble) species. High resolution 13C and 15N solid state NMR spectroscopies allow the assessment of the chemical changes occurring in the macromolecule upon solubilization. 1H and 13C NMR solution spectra are discussed in light of the results obtained from the solid state spectra. Furthermore, the coordination properties of melanin have been investigated through 27Al NMR spectroscopy and proton relaxation enhancement studies of the paramagnetic gadolinium complex of melanin free acid. Through these experiments it has been possible to evaluate the molecular reorientational time tau R (and from it an estimated molecular weight close to 20 KDa) and the strength of the metal-macromolecule interaction.

- Akiu S, Fukuda M.
Recent trends of development of melanin control agents. Fragrance J 20:29-34, 1992.
Abstract : A review with 15 references on research on the mechanism of melanogenesis and its excretion in the skin, development of the evaluation methods for melanin control agents, and examples of development of new melanin control agents in cosmetic industry.

- Al-Kazwini AT, O'Neill P, Adams GE, Cundall RB, Junino A, Maignan J.
Characterization of the intermediates produced upon one-electron oxidation of 4-, 5-, 6- and 7-hydroxyindoles by the azide radical. J Chem Soc, Perkin Trans 2:657-661, 1992.
Abstract : One-electron oxidation of a series of monohydroxylated indoles (HI) by the azide radical in the pH range 5-9 was studied by using the technique of pulse radiolysis with spectrophotometric detection. One-electron oxidation of 4-, 5-, 6- and 7-hydroxyindoles gives the indoloxyl radicals, the optical absorption spectra of which are independent of pH (5-9). It is confirmed, using the N(1)-Me substituted analog of 6-HI, that deprotonation of the resulting radical cation of the hydroxyindoles occurs preferentially from the hydroxy group to yield the corresponding indoloxyl radical. Such deprotonation would be consistent with the resulting indoloxyl radical having a low pKa. With the exception of 4-HI, the indoloxyl radicals decay bimol. in the dose/pulse range of 1-30 Gy to yield semipermanent products (2k = 2-4 times 109 dm3 mol-1 s-1). With 4-HI, the decay of the indoloxyl radical changes from second-order to first-order kinetics on lowering the dose/pulse. At 1 Gy/pulse, the first-order kinetics are dependent upon the concentration of 4-HI. The second-order rate constant for reaction of the indoloxyl radical with 4-HI was 4.8 times 107 dm3 mol-1 s-1. The decay of these semipermanent products from the indoloxyl radicals is first order and depends upon the concentration of azide. The second-order rate constants determined for this reaction depend markedly on the hdyroxyindole used (k = 159-821 dm3 mol-1 s-1). The semipermanent product arising from the bimol. decay of the indoloxyl radicals is discussed in terms of the formation of reactive quinone-methides and/or -imines.

- Al-Kazwini AT, O'Neill P, Cundall RB, Adams GE, Junino A, Maignan J.
Direct observation of the reaction of the quinonemethide from 5,6-dihydroxyindole with the nucleophilic azide ion. Tetrahedron Lett 33:3045-3048, 1992.
Abstract : Pulse radiolysis of aqueous solutions of 5,6-dihydroxyindole(I) and azide ion oxidizes I to the corresponding quinonemethide. The quinonemethide was found to decay by first-order kinetics; the rate is dependent on the concentration of azide up to 0.1 mol dm-3; at higher concentrations of azide, the first-order rate for loss of the quinone methide is independent of [azide]. The rate const. for interaction of the methide with azide was >5 x 102 dm3 mol-1 s-1. The quinonemethide is in equilibrium with its tautomer, the indoloquinone, which interacts preferentially with the azide ion. At high [N3-], the rate detg. step for the loss of the quinonemethide is tautomerization of the methide so that the disappearance of the methide becomes independent of the concentration of [N3-]. The driving force for the azide interaction is electrocyclic ring closure of the substituted intermediate. From comparison with the corresponding reactions of the quinonemethide from 5-methoxy-6-hydroxyindole, it is inferred that the indoloquinone of Iis involved in the pathways to melanin formation.

- Aroca P, Solano F, Salinas C, Garcia-Borron JC, Lozano JA.
Regulation of the final phase of mammalian melanogenesis. The role of dopachrome tautomerase and the ratio between 5,6-dihydroxyindole-2-carboxylic acid and 5,6-dihydroxyindole. Eur J Biochem 208:155-163, 1992.
Abstract : The regulation of the final steps of the melanogenesis pathway, after L-2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome) formation, is studied. It is shown that both tyrosinase and dopachrome tautomerase are involved in the process. In vivo, it seems that tyrosinase is involved in the regulation of the amount of melanin formed, whereas dopachrome tautomerase is mainly involved in the size, structure and composition of melanin, by regulating to the incorporation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) into the polymer. Moreover, using L-3,4-dihydroxyphenylalanine (dopa) and related compounds, it was shown that the presence of dopachrome tautomerase mediates an initial acceleration of melanogenesis since L-dopachrome is rapidly transformed to DHICA, but that melanin formation is inhibited because of the stability of this carboxylated indole compared to 5,6-dihydroxyindole (DHI), its decarboxylated counterpart obtained by spontaneous decarboxylation of L-dopachrome. Using L-dopa methyl ester as a precursor of melanogenesis, it is shown that this carboxylated indole does not polymerize in the absence of DHI, even in the presence of tyrosinase. However, it is incorporated into the polymer in the presence of both tyrosinase and DHI. Thus, this study suggests that DHI is essential for melanin formation, and the rate of polymerization depends on the ratio between DHICA and DHI in the medium. In the melanosome, this ratio should be regulated by the ratio between the activities of dopachrome tautomerase and tyrosinase.

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Melanin-related biochemistry of IGR 1 human melanoma cells. Melanoma Res 1:5-13, 1991.
Abstract : Cultured melanoma cells have been of great value in the study of pigment metabolism. IGR 1 human melanoma cells, established by Dr Christian Aubert, produce melanin in large quantities. These cells have been used for isolation of human tyrosinase which enzyme has not previously been obtained in a pure form. IGR 1 cells contain large amounts of 5-S-cysteinyldopa which is the quantitatively most important catecholic amino acid. This review deals with the metabolism of dopa, cysteinyldopa, glutathionyldopa, cysteine and glutathione, compounds of central importance in pigment metabolism. The information available on tyrosinase, catecholic compounds and on thiols in IGR 1 melanoma cells makes these cells most suitable for further investigation of the metabolism of human melanoma cells.

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The hydroxylation of phenylalanine and tyrosine: a comparison with salicylate and tryptophan. Arch Biochem Biophys 296:521-529, 1992.
Abstract : The hydroxylation of phenylalanine by the Fenton reaction and gamma-radiolysis yields 2-hydroxy-, 3-hydroxy-, and 4-hydroxyphenylalanine (tyrosine), while the hydroxylation of tyrosine

results in 2,3- and 3,4-dihydroxyphenylalanine (dopa). Yields are determined as a function of pH and the presence or absence of oxidants. During gamma-radiolysis and the Fenton reaction the same hydroxylated products are formed. The final product distribution depends on the rate of the oxidation of the hydroxyl radical adducts (hydroxycyclohexadiene radicals) relative to the competing dimerization reactions. The pH profiles for the hydroxylations of phenylalanine and tyrosine show a maximum at pH 5.5 and a minimum around pH 8. The lack of hydroxylated products around near pH 8 is due to the rapid oxidation of dopa to melanin. The relative abilities of iron chelates (HLFe(II) and HLFe(III) to promote hydroxyl radical formation from hydrogen peroxide are nitrilotriacetate (nta) greater than ethylenediaminediacetate (edda) much greater than hydroxyethylethylenediaminetriacetate greater than citrate greater than ethylenediaminetetraacetate greater than diethylenetriaminepentaacetate greater than adenosine 5'-triphosphate greater than pyrophosphate greater than adenosine 5'-diphosphate greater than adenosine 5'-monophosphate. The high activity of iron-nta and -edda chelates is explained by postulating the formation of a ternary Fe(III)-L-dopa complex in which dopa reduces Fe(III). The hydroxylations of phenylalanine and tyrosine are similar to that of salicylate (Z. Maskos, JD. Rush and WH. Koppenol, 1990, Free Radical Biol Med 8:153-162) and tryptophan (preceding paper) in that oxidants augment the formation of hydroxylated products by catalyzing the dismutation of hydroxyl radical adducts to the parent compound and a stable hydroxylated product. A comparison of salicylate and the amino acids tryptophan, phenylalanine, and tyrosine clearly shows that salicylate is the best indicator of hydroxyl radical production.

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Melanin is a double-edged sword. Photobiol Sci Its Appl, Proc Int Congr Photobiol, 10th Meeting, 1988, 873-86 (Riklis E., ed), Plenum, New York, NY, 1991.
Abstract : Melanin is known to photoprotect by phys. absorption/scattering of UV and by electron transfer/free radical scavenging. In this work, it is shown that melanin can also facilitate potentially harmful redox reactions in vitro. By kinetic spectrometry and O2 uptake, it is shown that both synthetic eumelanin (Sigma) and Sepia melanin extd. with cuttlefish markedly accelerate the tyrosinase-catalyzed oxygenation of the cytotoxic phenol p-hydroxyanisole MMEH to the cytotoxic 4-methoxy-1,2-benzoquinone. These studies indicate that a substrate-melanin complex transfers electrons to tyrosinase to regenerate Cu(I) necessary for reduction of mol. O2. Prior irradiation of melanin with solar-simulating UV results in evanescent changes in the kinetics of quinone formation which are consistent with reversible photooxidation of the melanin. The latter effect vis-a-vis photoprotection is ambivalent at best. Thus, melanin protects with one hand, and, perhaps unwittingly, may place surrounding cells at risk with the other.

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Melanin, nature's own sunscreen polymer. Cosmet Pharm Appl Polym, Proc Am Chem Soc Symp Polym Cosmet Pharm Appl, Meeting Date 1990, 83-94. (Gebelein CG, Cheng TC, Yang VCM, eds). Plenum, New York, NY, 1991.
Abstract : Melanin is a colored polymer widely distributed in species as diverse as mushroom, squid, sturgeon and chicken. In man, it is produced within the epidermis as a response to UV radiation injury. It protects the skin because it absorbs a wide range of UV and visible light. However, it has never been used as a sunscreen because (a) it was not available in common quantities and (b) it is difficult to formulate. Biosynthetic melanin, produced by genetic engineered organisms, was entrapped in a microsponge polymeric system and formulated in a cream base containing also regular sunscreens to boost the UVB absorbance. When this formulation was tested in humans, melanin provided better UVA protection than any other sunscreen available and at a lower concentration. Furthermore, studies conducted with radioactive melanin showed no penetration through human skin, thereby supporting its safety.

- Nappi AJ, Vass E, Carton Y, Frey F.
Identification of 3,4-dihydroxyphenylalanine, 5,6-dihydroxyindole, and N-acetylarterenone during eumelanin formation in immune reactive larvae of Drosophila melanogaster. Arch Insect Biochem Physiol 20:181-191, 1992.
Abstract : 3,4-Dihydroxyphenylalanine, 5,6-dihydroxyindole, and N-acetylarterenone were detected

by electrochemical methods in the hemolymph of immune reactive larvae of D. melanogaster following parasitization by the wasp Leptopilina boulardi. Detns. of the catechols were made after separation by reverse phase, ion-pairing HPLC with electrochemical detection. The presence of 5,6-dihydroxyindole unequivocally establishes the eumelanin pathway in the defense response of Drosophila, and confirms previous investigations which have implicated certain catecholamine metabolizing enzymes in insect immunity. The occurrence of N-acetylarterenone, a derivative of the principal sclerotizing agent N-acetyldopamine, verifies the existence and proposed involvement of quinone methide isomerase in the regulation of catecholamine metabolism, and suggests that the cellular capsule formed by Drosophila in immune reactions against parasites is most likely a composite of both eumelanin and sclerotin. The absence of 3,4-dihydroxyphenylacetic acid in hemolymph samples from immune reaction hosts suggests that during parasitization certain catecholamines and metabolic precursors may be re-employed in alternate pathways, some of which may be used in defense reaction.

- Pawelek JM.
After dopachrome. Reply to comments Pigm Cell Res 4:256, 1991.
Abstract : A polemic in response to F. Solano et al. (ibid., 255).

- Solano F, Garcia-Borron JC, Lozano JA, Aroca P.
After dopachrome. Comments. Pigm Cell Res 4:255, 1991.
Abstract : A polemic clarifying the authors' ideas about the nature of DHICA-melanins as presented in a review by JM. Pawelek (ibid. 4(2), 53-62).

- Tosk JM, Holshouser BA, Aloia RC, Hinshaw DBJ.
Effects of the interaction between ferric iron and L-dopa melanin on T1 and T2 relaxation times determined by magnetic resonance imaging. Magn Reson Med 26:40-45, 1992.
Abstract : T1 and T2 relaxation times of agar phantoms containing L-dopa melanin and Fe3+ were measured under MRI conditions. Fe3+ shortened T1 and T2 relaxation times. Melanin influenced relaxation times only in the presence of Fe3+; thus, contrast in MR images of the basal ganglia may depend upon levels of both paramagnetic iron and neuromelanin.

- Tsukamoto K, Palumbo A, D'Ischia M, Hearing VJ, Prota G.
5,6-Dihydroxyindole-2-carboxylic acid is incorporated in mammalian melanin. Biochem J 286:491-495, 1992.
Abstract : The role of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) in the biosynthesis of melanins has been studied by using the incorporation of specifically radiolabelled melanogenic precursors into melanins formed by melanocytes growing in vitro and in vivo. Extracts of mouse melanocytes and intact viable melanocytes were found to incorporate into melanin from 25% to more than 60% of [1-14C]tyrosine. Melanins from melanoma tumours grown in mice were radiolabelled with 3,4-dihydroxy[1-14C]phenylalanine, purified and chemoselectively decarboxylated. Determination of the 14CO2 evolved showed that at least 20% of the precursor incorporated in vivo retains the label in the form of non-aminoacidic aromatic-type carboxyl groups. These results provide the first unambiguous demonstration that DHICA is incorporated in physiologically relevant amounts in mammalian melanins.

2. Biology of pigment cells and pigmentary disorders

- Albig J, Gollnick H, Detmar M, Orfanos CE.
Linear hyperpigmentation caused by bleomycin. Hautarzt 43:376-379, 1992.
Abstract : We report on an uncommon but characteristic cutaneous side-effect of bleomycin. A 52-year-old woman being treated for carcinoma of the cervix developed linear hyperpigmentation in wheals in the lumbosacral region, the lateral thorax and above the elbow. The skin lesions appeared during the fourth cycle of chemotherapy with bleomycin. Histologically, incontinence of melanin,

focal parakeratosis and a lymphocytic infiltrate with epidermotropism were prominent. By electron microscopic examination metabolically highly active melanocytes were found, with increased number of melanosomes at all stages of maturation and deposits of extracellular melanin in the underlying dermis. The epidermal keratinocytes were unchanged.

- Anavi Y, Mintz S.
Unusual physiologic melanin pigmentation of the tongue. Pediatr Dermatol 9:123-125, 1992.
Abstract : A patient had extensive congenital oral hyperpigmentation of the tongue. The clinical and histologic features set this case apart from any well-delineated disease. Clinically, the congenital onset, the appearance of large black-brownish lesions, the lack of associated systemic abnormalities, and the histologic findings of prominent deposition of melanin in the basal layer support the diagnosis of physiologic melanosis. The macular lesions of the tongue represent discrete depositions of melanin and exemplify soft tissue pigmentation of developmental origin.

- Borovansky J, Vedralova E, Hach P.
An estimate of melanosome concentration in pigment tissues. Pigment Cell Res 4:222-224, 1991.
Abstract : Concentration of melanosomes in various tissues has been unknown because of the impracticability of their direct quantification. Using an indirect approach comprising the estimation of melanin both in freeze-dried tissue samples and in isolated melanosomes, we obtained data on the amount of melanosomes in various pigment tissues. The concentrations of melanosomes found in the tissues were relatively high, not only reflecting the dark color of pigment tissues but also explaining their capacity to perform various functions ascribed to the presence of melanin.

- Buhl AE, Kawabe TT, MacCallum DK, Waldon DJ, Knight KA, Johnson GA.
Interaction of minoxidil with pigment in cells of the hair follicle: an example of binding without apparent biological effects. Skin Pharmacol 5:114-123, 1992.
Abstract : To identify minoxidil target cells in hair follicles we followed the uptake of radiolabeled drug in mouse vibrissae follicles both in vitro and in vivo. Autoradiography showed that both 3H-minoxidil and 3H-minoxidil sulfate accumulated in the differentiating epithelial matrix cells superior to the dermal papilla, a distribution similar to that of pigment. Minoxidil localized in melanocytes, melanocyte processes, and areas of greater melanin concentrations within the epithelial cells. Although uptake of minoxidil was significantly less in unpigmented follicles, the drug stimulated proliferation and differentiation of both pigmented and unpigmented follicles. Labeled minoxidil bound to Sepia melanin and was displaced with unlabeled minoxidil and other electron donor drugs. This interaction with melanin acts as a targeting mechanism of minoxidil to pigmented hair follicles but has no apparent functional significance in hair growth. This work illustrates how measurement of drugs in hair may be biased by pigmentation.

- Carbonell AL, Boya J, Garcia-Maurino JE.
Presence of melanin in normal human Schwann cells. Histol Histopathol 7:329-332, 1992.
Abstract : The presence of melanin granules in Schwann cells of unmyelinated nerve fibres in the normal skin of a black woman is demonstrated by electron microscopy. Pathological conditions associated with the differentiation ability of Schwann cells for melanogenic are reviewed. This capacity may be due to the common origin of Schwann cells and melanocytes in the neural crest.

- Cegarra J, Gacen J, Cayuela D, Caro M.
Action of various sequestering agents in the depigmentation of alpaca hair. Rev Quim Text 104:42-43, 46-48, 50-52, 1991.
Abstract : Max. sequestering activity for Fe was attained by Na nitriloacetate (NTA), EDTMP, and N-oxyaminotrimethylenephosphonic acid (I) in depigmentation of alpaca, when the pH of the bath was 5-6. There was removal of Fe from the non-melanin fractions of the fibers to prevent damage by interactions of Fe with bleaching agents. The alpaca hair was treated with H3PO3, FeSO4, and mordants; subsequently, the sequestering agent was added and treatment was continued for 20 min at 80 degree.The alpaca was then bleached with H2O2/Na4P2O7.10H2O for 1 at 70 degree. The activity of Na NTA was similar to that of EDTMP and slightly better than that of I. The toughness of

treated alpaca fibers was lower in material treated with Na NTA than with the phosphonate agents.

- Fleegler EJ.
A surgical approach to melanonychia striata. J Dermatol Surg Oncol 18:708-714, 1992.
Abstract : Longitudinal pigmented streaks - melanonychia striata longitudinalis - are discussed from the perspective of a difficult diagnostic problem. These must be differentiated as early as possible from melanoma. The history of this subject and evaluation of patients in the context of various populations are reviewed. Difficult decisions and technical aspects of the approach are outlined. Case presentations contrast multiple different subungual pigmented lesions that enter into the differential diagnosis of these tumors. A larger number of lesions that make up this differential diagnosis are also discussed in addition to the cases. Appropriate biopsy is reviewed. The potential deformities from the biopsy are contrasted with dangers that are associated with some subungual pigmented tumors.

- Fuqua WC Jr.
Melanin biosynthesis in the marine bacterium Shewanella colwelliana: genetic and biochemical characterization. Univ Microfilms Int, Order No. DA9133076 From: Diss. Abstr Int B 1991, 52(6), 2891.

- Gordon L, Peacocke M, Gilchrest BA.
Induction of c-fos but not c-myc in S-91 cells by melanization signals. J Dermatol Sci 3:35-41, 1992.
Abstract : Synthesis of the pigment melanin is a complex differentiated function performed by pigment cells in response to a variety of stimuli. The possible roles of the proto-oncogenes c-fos and c-myc in the control of pigmentation were studied using subconfluent, actively proliferating Cloudman S-91 murine melanoma cells stimulated to synthesize melanin by melanocyte stimulating hormone (MSH) or forskolin. Stimulation caused a significant increase in melanin synthesis when compared to control cells, but had no effect on cell growth. Northern analysis of total cellular RNA demonstrated rapid, transient induction of c-fos mRNA as early as 30 min after stimulation with MSH or forskolin. In contrast, there was no effect on the high constitutive expression of c-myc in these actively proliferating cells. These data strongly suggest that the induction of c-fos mRNA is an early genetic event in stimulation of melanin synthesis and thus this proto-oncogene may play a major role in the regulation of this differentiated function, as reported for other forms of cellular differentiation. In contrast, c-myc expression is unaffected and instead correlated with cellular proliferative capacity. These results are consistent with the hypothesis that the down-regulation of c-myc frequently observed during cell differentiation is not a necessary event, but rather reflects an associated decrease in cell growth rate. The S-91 melanoma system appears to provide a convenient model for study of the regulation for a single, well defined differentiated function that is independent of growth rate.

- Gratton MA, Wright CG.
Hyperpigmentation of chinchilla stria vascularis following acoustic trauma. Pigment Cell Res 5:30-37, 1992.
Abstract : This report describes morphological alterations of the chinchilla stria vascularis seen 30 days after exposure to impulse noise. The observed changes included a dramatic increase in strial melanin content which occurred in 7 of 36 animals exposed to electronically synthesized impluses presented in various temporal patterns at either 135 or 150 dB peak SPL. In these animals, densely pigmented areas of stria 1.5 to 3 mm in length were found in the basal cochlear turn. Light and electron microscopic study revealed that these areas contained large numbers of melanin granules situated primarily in pale-staining cells of the middle layer of the stria. Unlike the pigment granules present in normal chinchilla stria, the melanosomes found in the noise-exposed material clearly showed ultrastructural features characteristic of eumelanin. Melanin granules were also observed in marginal and basal cells of the noise-exposed stria. In some cases, pigment granules which had apparently been expelled from the marginal cells were present in the endolymphatic space beneath Reissner's membrane and on the strial surface. These findings support the view that the melanin-bearing cells of the inner ear are capable of markedly increased activity in response to stressful conditions.

- Gross A, Tapia FJ, Mosca W, Perez RM, Briceno L, Henriquez JJ, Convit J.
Mononuclear cell subpopulations and infiltrating lymphocytes in erythema dyschromicum perstans and vitiligo. Histol Histopathol 2:277-283, 1987.
Abstract : Erythema dyschromicum perstans (EDP) and vitiligo are two cutaneous pigmentary dermatoses of unknown etiology. In the present study, the leukocyte infiltrates in the affected skin of EDP and vitiligo patients were studied using the avidin-biotin (ABC) immunoperoxidase technique and monoclonal antibodies which recognise the following mononuclear cell subgroups: T-suppressor/cytotoxic (CD8-Leu-2), T-helper (CD4 = OKT4), T-suppressor + macrophages (Leu-15), Pan T (CD3 = Leu-4), macrophages (Leu-M3) and Langerhans cells (CD1 = Leu-6), and other cellular markers such as Ia antigens and the Interleukin-2 receptor (CD25 = TAC). The immunocytochemical analysis showed a selective accumulation of CD3+, CD8+, Leu-15-, T-cytotoxic cells in the epidermis of both EDP and early lesions of vitiligo. In addition, an increase in the number of epidermal Langerhans cells (CD1+) was observed in some cases of EDP and vitiligo. The CD4/CD8 ratios in affected and uninvolved skin for both disorders were not significantly different, although values lower than unity were only observed in the infiltrates of affected skin. Ia antigen positivity was observed in the dendritic cells of the dermis and epidermis, as well as in most of the lymphoid cells within the infiltrates for both diseases. Macrophages (Leu-M3) in EDP dermal infiltrates were generally found adjacent to extracellular melanin pigment. Lymphocytes expressing TAC (CD25) surface antigens were also present in the dermal infiltrates. These morphological observations suggest a possible immune cell participation in the dyschromia of such cutaneous disorders.

- Hou L, Takeuchi T.
Differentiation of extracutaneous melanocytes in embryos of the turtle, Trionyx sinensis japonicus. Pigment Cell Res 4:158-162, 1991.
Abstract : The present study investigates the mode of differentiation of neural crest-derived melanocytes in the embryos of the soft-shell turtle, Trionyx sinensis japonicus. DOPA reaction-positive melanoblasts were first detected in 10-day-old embryos. Melanocyte differentiation in terms of pigmentation takes place from the day 16 of development. Melanin pigments were found in the dorsal integument as well as in various extracutaneous tissues such as skeletal muscle, dorsal aorta, peritoneum, blood vessels, choroid, lung, bone marrow, fat tissues and in the connective tissue of the nose. These results suggest the presence of a specific environmental regulation of the melanoblast differentiation in the soft-shell turtle.

- Ito A, Tanaka C, Takeuchi T, Mishima Y.
Glucocorticoid stimulates melanogenesis and tyrosinase gene expression in B16 melanoma cells. Pigment Cell Res 4:247-251, 1991.
Abstract : Effects of dexamethasone on melanogenesis and tyrosinase mRNA levels were determined in B16/F10 melanoma cells. Melanin content of B16 cells increased in a dose-dependent manner by the addition of dexamethasone to the culture medium. After 72 hr exposure, dexamethasone (10(-6) M) produced a 2.4-fold increase in melanin content. Northern blot analysis revealed that tyrosinase mRNA level also increased by the addition of dexamethasone to the culture medium. After 24 hr exposure, dexamethasone (10(-6) M) caused a 1.8-fold increase in tyrosinase mRNA levels. A tumor promoter, 12-O-tetradecanoyl phorbol-13-acetate (TPA) decreased tyrosinase mRNA level at 30 nM concentration. Dexamethasone antagonized this TPA-mediated decrease in tyrosinase mRNA. It is suggested that glucocorticoids are involved in the regulation of tyrosinase activity at the transcriptional level.

- Johnston D, Orlow SJ, Levy E, Bystryn JC.
Induction of B16 melanoma melanogenesis by a serum-free synthetic medium. Exp Cell Res 201:91-98, 1992.
Abstract : Cultured murine B16 melanoma cells normally grow as spindle-shaped cells firmly attached to tissue culture flasks. Pellets obtained from harvested B16 melanoma cells are white to grey in color. When the same cells were grown in synthetic, serum-free AIM V medium, cellular morphology and pigmentation were radically altered. Within 3 days of subculture in AIM V, cells rounded up and grew in clusters in suspension. Melanin content increased to greater than 30 times and tyrosinase

activity was found to be 10-50 times higher in cells grown in AIM V medium compared to those cultured in normal medium. A concomitant increase in the level of immunoreactive tyrosinase was also induced. The individual growth factors and hormones present in AIM V medium were examined to determine which component(s) stimulates melanogenesis. Only those cells grown in the presence of 2.5% human albumin were stimulated to synthesize melanin. These findings suggest that albumin, or a component associated with albumin, has a major effect upon the regulation of melanogenesis in these cells.

- Kameyama K, Morita M, Sugaya K, Nishiyama S, Hearing VJ.
Treatment of reticulate acropigmentation of Kitamura with azelaic acid. An immunohistochemical and electron microscopic study. J Am Acad Dermatol 26:817-820, 1992.
Abstract : No successful therapy has been reported for reticulate acropigmentation of Kitamura, which is an autosomal dominant dermatosis. We treated a patient with 20% azelaic acid ointment. Within several weeks the pigmentation was remarkably decreased and no side effects were observed. Histologic examination revealed an increased number of dopa-positive melanocytes. These cells reacted strongly to staining with antityrosinase antibody or antityrosinase-related protein antibody. Electron microscopic findings showed many melanosomes within melanocytes, keratinocytes, and melanophages. These findings suggest that the hyperpigmentation of reticulate acropigmentation of Kitamura is the result of an excess amount of melanin production caused by activation of melanocytes in the basal layer.

- Kawaguchi Y.
The inhibitory effects of licorice extracts (flavonoids) on melanogenesis. I. In vitro studies. Nippon Hifuka Gakkai Zasshi 102:679-688, 1992.
Abstract : Melanogenesis was assayed by 14C-thiouracil uptake into B-16 melanoma cells and it was inhibited by flavonoids-containing Glycyrrhiza glabra extracts (LE-I) with dose-dependent tendency. LE-I did not affect DNA synthesis of B-16 cells in the range of concentration used. Electron microscopic examination on the LE-I treated B-16 melanoma cells showed that the no. of stage-II, -III, and -IV melanosomes were reduced, and Golgi areas and melanosomes were dopa-negative. By electrophoretic analysis, T1 and T3 tyrosinase of LE-I treated B-16 melanoma cells was decreased. The split-dopa reaction of human skin incubated with LE-I showed that human melanocytes were dopa-negative. These results indicate that LE-I has inhibitory effects on tyrosinase activity in vitro. However, to understand the inhibitory mechanism of this extract, further detailed studies are necessary.

- Kawaguchi Y, Goh K, Kawa Y, Kashima M, Mizoguchi M.
The inhibitory effects of licorice extract (flavonoids) on melanogenesis. II. In vivo studies. Nippon Hifuka Gakkai Zasshi 102:689-694, 1992.
Abstract : The in vivo effects of flavonoids-containing licorice extract (LE-1) on pigmentation was studied to establish the clinical and cosmetic values of its depigmenting effects. Brownish guinea pig skin was depigmented and epidermal melanin granules and dopa-positive cells were reduced by LE-I and hydroquinone (HQ) on UVB- and psoralen plus UVA-induced pigmentation. The human skin was depigmented also by LE-I on UVB-induced pigmentation. From these results it is concluded that LE-I has depigmenting effects in vivo.

- Konohana A.
Blue-gray pigmentation in a patient receiving doxorubicin. J Dermatol 19:250-252, 1992.
Abstract : A 64-year-old Japanese female who was treated for hepatocellular carcinoma with doxorubicin developed diffuse blue-gray pigmentation of the face, fading out on the upper trunk. Skin biopsy revealed many melanin granules in the upper dermis. It is believed that the pigmentation was induced by doxorubicin.

- Lacour JP.
Culture of human melanocytes. Its contribution to the knowledge of melanocyte physiology. Pathol Biol (Paris) 40:114-120, 1992.

Abstract : Culture techniques for normal human melanocytes have been developed over the last ten years. This in vitro model for studying pigment-producing cells can be expected to provide major advances in the knowledge of cell-to-cell and cell-to-matrix interactions, melanocyte and melanin biology, pathophysiology of pigment disorders, and malignant melanomas. Melanocyte cultures have already shed new light on keratinocyte-melanocyte interactions within the epidermal melanin unit by showing that keratinocytes produce "melanotrophic factors" which modulate growth, melanin production, and dendricity of melanocytes. Melanocyte cultures also enable in vitro studies of melanocyte responses to ultraviolet radiations and of the biologic messengers involved in these responses. Lastly, they provide a means for investigating endocrine and paracrine mechanisms involved in the regulation of melanogenesis, including the role of melanotropins, estrogens and vitamin D. Improved knowledge of the molecular biology of melanocytes provides hope for rapid advances in the understanding of tyrosinase and posttyrosinase regulation of melanogenesis. Lastly, melanocyte cultures can be expected to find useful applications in the pathophysiologic study of pigment disorders and of pharmacologic modulation of skin pigmentation.

- Maeda K, Fukuda M.
In vitro effectiveness of several whitening cosmetic components in human melanocytes. J Soc Cosmet Chem 42:361-368, 1991.
Abstract : The inhibitory action of arbutin, kojic acid, and ascorbic acid on tyrosinase activity in human melanocytes was compared. The substances are active whitening cosmetic components. Hydroquinone was used as a positive control. The depigmenting effect of linoleic acid, which has been reported to inhibit melanin synthesis, was compared with those of arbutin, kojic acid, and ascorbic acid. Human melanocytes were cultured with each agent in multiwell plates for three days, and the tyrosine activity was assayed using L-DOPA as a substrate. In addition, cell viability of three-day cultures was evaluated by the MTT test. Arbutin dose-dependently reduced tyrosinase activity at final concentrations between 0.01 mM and 1.0 mM, at which no change in cell viability was seen. This action was about 1/100 that of hydroquinone, and was stronger than that of kojic acid and ascorbic acid. Linoleic acid did not reduce tyrosinase activity at non-cytotoxic ranges. Furthermore, at concentrations of 0.5 mM, the amount of melanin was reduced significantly by arbutin.

- Marchesi L, Naldi L, Di Landro A.
Segmental lentiginosis with "jentigo" histologic pattern. Am J Dermatopathol 14:323-327, 1992.
Abstract : We report a case of segmental lentiginosis (unilateral lentiginosis), that is, asymmetric distribution of lentigines on one side of the body, in a 23-year-old woman. Lesions involved the right side of the face and the cervical region, mostly within the area of division of the trigeminal nerve. Histologic examination disclosed a lentiginous pattern as well as some nests of melanocytes at the dermal-epidermal junction (so-called jentigo pattern). Similar cases have been described in the literature under the term "zosteriform lentiginous nevus," which in our opinion makes for confusion since the same term has also been used to describe cases that fit the diagnostic criteria for speckled lentiginous nevus (nevus spilus).

- Matsui S, Tanabe T, Furuichi M, Yoshimatsu T, Kitajima C.
Reduction of black lines in the muscle of cultured red sea bream and improvement of the body color. Nippon Suisan Gakkaishi 58:1459-1464, 1992.
Abstract : The flesh quality of cultured red sea bream is distinguished from wild red sea bream by the body color and black lines in the muscle. The part where black lines appear and their properties were clarified by histochemical analysis. Furthermore, effects of sun-shade in rearing under different shade rates were examined for the purpose of protecting the surface of the body from melanins accumulation and removing black lines from the muscle. Black lines were observed along the periphery of the blood vessels, while dark green staining with ferric ferricyanide and decoloration with 40% peracetic acid showed the presence of melanin. Unlike melanophores in the cutis, those in the black lines didn't respond either to KCl or to NaCl. The brightness (L value) of the body color of the sun-shaded specimens detected by using a color meter was similar to that of wild red sea bream, and the d. of black lines decreased with 17-day sun-shade at a rate of more than 90% shade.

The wild red sea bream maintained body color during the experimental sun-shade period. Of UV and visible rays, shading of the latter was more effective in the removal of black lines. It has been definitely shown by these results that sun-shade of more than 17 days and a rate of 90% shade is effective in the improvement of flesh quality of cultured red sea bream.

- Mihara M, Nakayama H, Aki T, Inoue T, Shimao S.
Cutaneous nerves in cafe au lait spots with white halos in infants with neurofibromatosis. An electron microscopic study. Arch Dermatol 128:957-961, 1992.
Abstract : Although two cardinal skin manifestations of neurofibromatosis are cutaneous neurofibromas and cafe au lait spots, the pathogenesis of cafe au lait spots are very poorly known compared with that of cutaneous neurofibromas. Thus, the cafe au lait spots in two Japanese infants were clinically, histologically, and electron-microscopically investigated. Some of the cafe au lait spots in the mongolian spots were surrounded by white halos. Histologically, in the cafe au lait spots, the epidermal basal cells had abundant melanin pigment, but macromelanosomes were not seen throughout the epidermis. In the white halo, the epidermal basal cells had a small amount of melanin pigment. Electron microscopically, the cafe au lait spots and their white halos had many subepidermal and intraepidermal nerves that belonged to free nerve endings. All the cutaneous nerves were mature. Some of the intraepidermal nerves had partially or completely naked axons that contacted tightly with the cytomembranes of the basal keratinocytes. Some of the axons in the subepidermal nerves showed degenerative changes only in the white halos. No ultrastructural pathologic changes were observed in the melanocytes, the epidermal keratinocytes, or melanosomes in those cells in the cafe au lait spots and their white halos; also, dermal melanocytes were absent in the both areas. The increase of the cutaneous nerves and the absence of dermal melanocytes in the cafe au lait spots and their white halos may be considered as characteristic histologic cutaneous findings in infants with neurofibromatosis. However, no evidence indicates that the cutaneous nerves may participate closely in the pathogenesis of the white halos.

- Otwell WS, Iyengar R, McEvily AJ.
Inhibition of shrimp melanosis by 4-hexylresorcinol. J Aquat Food Prod Technol 1:53-65, 1992.
Abstract : 4-Hexylresorcinol is an effective processing aid for the inhibition of shrimp melanosis (blackspot) in both lab. and field trials. The compound is a potent inhibitor of shrimp polyphenol oxidase. A one minute dip in 50 ppm 4-hexylresorcinol in sea water is sufficient to inhibit melanosis for up to 14 days of ice storage. The inhibitor has no negative effects on shrimp organoleptic quality and residual levels on treated product are typically .ltoreq.1 ppm (Iyengar R et al, 1991). 4-Hexylresorcinol can be used in traditional dip procedures by simple substitution for sulfiting agents and requires no changes in downstream processing of the shrimp product.

- Park S, Albert DM, Bolognia JL.
Ocular manifestations of pigmentary disorders. Dermatol Clin 10:609-622, 1992.
Abstract : Disorders of pigmentation can result from either an abnormal number of melanocytes, as in nevus of Ota and vitiligo, or an abnormal amount of melanin production, as in albinism. Melanin-producing cells are found in the skin, mucous membranes, uveal tract, and retinal pigment epithelium of the eye and the stria vascularis of the inner ear. Thus, many of the hereditary or congenital pigmentary disorders of the skin are associated with similar pigmentary abnormalities in the eye, such as iris heterochromia or changes in pigmentation of the fundus; however, more commonly, the associated eye finding is a defect in ocular motility, i.e., strabismus and nystagmus, suggesting a concomitant defect in neurologic development. In albinos, the observed neurologic abnormality in the visual pathway and foveal hypoplasia are hypothesized to be related directly to the lack of melanin in the pigment epithelium during development. In acquired disorders of pigmentation, in particular, vitiligo, Vogt-Koyanagi-Harada syndrome, and onchocerciasis, there is a frequent association with uveitis, suggesting an inflammatory cause for the cutaneous pigmentary changes.

- Shereef PH.
Hypopigmented macules in leprosy--a histopathological and histochemical study of melanocytes.

Indian J Lepr 64:189-191, 1992.
Abstract : Study of the number of melanocytes and amount of pigmentation in hypopigmented lesions and adjacent normal areas in 20 leprosy patients showed no differences in these parameters. It appears that hypopigmentation in leprosy lesions could be caused by defective transfer of melanin into keratinocytes.

- Tomita Y, Kondo Y, Ito S, Hara M, Yoshimura T, Igarashi H, Tagami H.
Menkes' disease: report of a case and determination of eumelanin and pheomelanin in hypopigmented hair. Dermatology 185:66-68, 1992.
Abstract : We report a male infant with Menkes' disease who showed, at the age of 3 months, slow growth, hair abnormalities such as pili torti and white hair, and low levels of serum copper and ceruloplasmin. The exceptionally bright portions of his hair contained eumelanin and pheomelanin at levels only half those of normal Japanese controls. After subcutaneous administration of copper-histidinate for 2 months, his scalp hair changed to dark brown.

- Zhang L, Yoshida T, Kuroiwa Y.
Stimulation of melanin synthesis of B16-F10 mouse melanoma cells by bufalin. Life Sci 51:17-24, 1992.
Abstract : Bufalin, which is one of prominent components of Chinese toad venom, was found to decrease the rate of cell proliferation of mouse melanoma clone B16-F10 cells and a concomitant stimulation of expression of its melanotic phenotype. The effect of bufalin on melanogenesis included stimulation of tyrosinase activity and increase of cellular melanin content. These effects became apparent after 48 hr exposure to 10(-4) M bufalin and increased thereafter. Other cardiotonic steroids, such as cinobufagin and ouabain, at the concentration of 10(-4) M for 6 days, also showed the stimulatory effect on melanin synthesis of B16-F10 cells, but not digitoxigenin.

3. MSH, MCH, other hormones, differentiation

- Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE.
The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 319:218-245, 1992.
Abstract : In addition to a nonadecapeptide homologous to the teleost melanin-concentrating hormone (MCH), the amino acid sequence predicted from a rat prepro-MCH (ppMCH) cDNA suggested that at least one (neuropeptide EI, or NEI), and possibly a second (NGE), additional neuropeptide may be encoded by this precursor. Cross-reactivity with epitopes of NEI or NGE can account for reported localization of alpha-MSH, rat CRF, and human GRF in rat dorsolateral hypothalamic neurons. We have used antisera raised against rat MCH and NEI in immunohistochemical studies at the light and electron microscopic levels, along with hybridization histochemical localization of ppMCH mRNA, to define the organization of this system. As expected, ppMCH mRNA is prominently expressed in cells in the lateral hypothalamic area and zona incerta. The MCH and NEI peptides were extensively colocalized in neurons in both of these areas. In addition, smaller cell groups in the olfactory tubercle and pontine tegmentum were also positively hybridized for ppMCH mRNA and immunostained for MCH and NEI. Fibers stained for MCH and NEI were similarly, and very broadly, distributed throughout the central nervous system in patterns that generally conformed with known projection fields of the lateral hypothalamic area and zona incerta. A differential distribution was seen in at least one region, the interanterodorsal nucleus of the thalamus, which contained a prominent terminal field stained for MCH but not NEI. At the electron microscopic level, MCH-stained perikarya displayed a prominent staining associated with the Golgi apparatus; this was not encountered in NEI-stained cells. Both peptides were distributed similarly in terminals in the lateral hypothalamic area and median eminence, with staining associated principally with dense-cored vesicles. The results suggest that ppMCH-derived peptides may serve as neurotransmitters or modulators of prominence in a surprisingly expansive projection field of incerto-hypothalamic neurons. The terminal distributions of this system seem most compatible with

functional roles in generalized arousal and sensorimotor integration, processes previously implicated as being subject to modulation by the lateral hypothalamic area.

- Checler F, Dauch P, Barelli H, Nahon JL, Vincent JP.
Hydrolysis of rat melanin-concentrating hormone by endopeptidase 24.11 (neutral endopeptidase). Biochem J 286:217-221, 1992.
Abstract : Melanin-concentrating hormone (MCH) is a cyclic peptide which behaves as an antagonist of the pituitary melanotropic hormone alpha-melanocyte-stimulating hormone in fishes. Cloning of the rat MCH cDNA precursor recently revealed the presence of an additional putative peptide named NEI. The present work examined the susceptibility of these novel peptides to hydrolysis by various purified exo- and endo-peptidases including endopeptidases 24.11 (NEP), 24.15, 24.16, angiotensin-converting enzyme, leucine aminopeptidase and carboxypeptidase A. NEP attacked MCH at three sites of the molecule with an apparent affinity of about 12 microM and a kcat. of 4 min-1. The first site of cleavage was at Cys-7-Met-8, i.e. within the peptide loop formed by the internal disulphide bridge. NEP could therefore be considered as an MCH-inactivating peptidase since the degradation products generated are probably devoid of biological activity. In contrast, NEI neither inhibited the degradation of the NEP chromogenic substrate glutaryl-Phe-Ala-Phe-p-aminobenzoate nor was susceptible to proteolysis by NEP. Unlike NEP, angiotensin-converting enzyme, endopeptidase 24.15 and endopeptidase 24.16 appeared totally unable to cleave MCH, whereas the peptide was readily degraded by aminopeptidase M and carboxypeptidase A.

- De Koning HP, Jenks BG, Scheenen WJJM, Balm PHM, Roubos EW.
Analysis of autofeedback mechanisms in the secretion of pro-opiomelanocortin-derived peptides by melanotroph cells of Xenopus laevis. Gen Comp Endocrinol 87:394-401, 1992.
Abstract : The possible existence of an autofeedback exerted by alpha-MSH or other POMC-derived peptides on melanotroph cells of the amphibian X. laevis has been investigated. alpha-MSH or its potent agonist [Nle4,D-Phe7]-alpha-MSH had no effect on the release of radiolabeled POMC-derived peptides or immunoreactive beta-endorphin from superfused neurointermediate pituitary lobes. Melanin-concentrating hormone, previously reported to have an alpha-MSH-like effect on melanophores, did not effect alpha-MSH secretion. Neurointermediate lobe superfusate, which contains a mixture of POMC-derived peptides, failed to affect the secretory activity of melanotrophs. Apparently, in X. laevis the secretory activity of melanotrophs is not under the control of short-term autofeedback mechanisms involving alpha-MSH or other POMC-derived peptides.

- Eberle AN, Drozdz R, Baumann JB, Girard J.
Receptor-specific antibodies by immunization with "antisense" peptides? Pept Res 2:213-220, 1989.
Abstract : Synthetic peptides whose sequences are specified by RNA complementary to the mRNA coding for peptide hormones have been reported to be useful antigens for the generation of receptor-specific antibodies. We have synthesized an eikositetrapeptide whose sequence corresponds to the complementary strand of the mRNA coding for the sequence of human ACTH(1-24). This "antisense" ACTH(1-24) peptide, "HTCAh," was coupled to bovine serum albumin or thyroglobulin prior to injection into rabbits. The complex proved to be very antigenic, inducing antisera of high titer and specificity. The antisera were tested in ACTH and MSH binding and bioassays, with or without prior purification of IgG molecules. None of the antisera displayed any effect in these assays, nor did they bind to blotted MSH/ACTH receptor protein from Cloudman S91 melanoma cells or to ACTH antibodies. The HTCAh peptide itself did not display measurable association to tritiated or iodinated ACTH(1-24), nor did it displace ACTH(1-24) in a receptor binding assay. However, the peptide bound to a low affinity site of mouse B16 melanoma cells which was independent of the MSH/ACTH binding site and induced melanin formation in these cells, but only at relatively high peptide concentration. Thus, in our hands, the antisense peptide approach using HTCAh as antigen did not lead to receptor-specific antibodies.

- Fric I, Lebl M, Hruby VJ.
Melanin concentrating hormone analogs: contraction of the cyclic structure. III. CD spectroscopic study. Collect Czech Chem Commun 57:614-620, 1992.

Abstract : A comparison of the CD spectra of melanin concentrating hormone (MCH) analogs differing in length but containing a disulfide ring of the same size reveals that a conformational change occurs upon elongating the peptide from 13 to 17 amino acid residues. The 5-17 fragments appear to prefer a beta-turn conformation, whereas the 1-17 full sequence peptides prefer alpha-helical conformations. Peptides containing 17-membered disulfide rings exhibit greater conformational adaptability than those containing a 26-membered disulfide ring. Spectral properties of the 23-membered disulfide ring in [Cys8]MCH8-17 and [Ala5,Cys8]MCH5-17 indicate that they do not possess highly ordered conformations.

- Johnson WC, Samaraweera P, Zuasti A, Law JH, Bagnara JT.
Preliminary biological characterization of a melanization stimulating factor (MSF) from the dorsal skin of the channel catfish, Ictalurus punctatus. Life Sci 51:1229-1236, 1992.
Abstract : A two step fractionation of conditioned media made from the darkly pigmented dorsal skin of the channel catfish, Ictalurus punctatus, has produced fractions that contain a melanization stimulating factor (MSF). Isolated neural tubes of Xenopus laevis embryos exposed to conditioned media and to specific fractions exhibit greater melanization (increased numbers of melanized cells and elevated percentages of melanized cells), a greater number of dendrites per melanized cell, and a greater number of emigrated neural crest cells than control neural tubes. The presence of MSF activity in the darkly pigmented dorsal integument suggests a role for a molecule or molecules in the development and maintenance of the dorsal/ventral pigment pattern of this piscine species and possibly of other vertebrates.

- Lunec J, Pieron C, Thody AJ.
MSH receptor expression and the relationship to melanogenesis and metastatic activity in B16 melanoma. Melanoma Res 2:5-12, 1992.
Abstract : In this study we have compared the effects of different pro-opiomelanocortin (POMC) peptides on melanogenesis and metastasis and their relationship to MSH receptor expression in B16F1 melanoma cells. All peptides, apart from beta-endorphin, increased melanogenesis and the order of potency was Nle4DPhe7-alpha-MSH greater than alpha-MSH greater than ACTH[1-39] greater than des-acetyl alpha-MSH greater than ACTH[1-24]. A similar order of potency was found for metastasis, except for ACTH [1-24], which had a relatively greater effect on metastasis. These findings suggest that the effects on melanogenesis and metastasis are mediated via the same receptor. The results of ligand binding studies also indicated the presence of a single receptor with a KD value for Nle4DPhe7-alpha-MSH of 62 +/- 16pM. This was consistent with crosslinking studies using [125I] Nle4DPhe7-alpha-MSH which produced a single 50-55 kD band on analysis by SDS-PAGE. However, the relative binding affinities of the different peptides, measured by displacement of [125I]-Nle4DPhe7-alpha-MSH, did not closely correlate with the relative potencies in stimulating melanogenesis and metastasis. This suggests that receptor activation and the subsequent biological response is not determined solely by binding affinity.

- Mangano FT, Fukuzawa T, Johnson WC, Bagnara JT.
Intrinsic pigment cell stimulating activity in the skin of the leopard frog, Rana pipiens. J Exp Zool 263:112-118, 1992.
Abstract : Consistent with the concept that specific pigment patterns of amphibians might result from the highly localized distribution of stimulators and inhibitors of pigment cell expression in the skin, the spot pattern of the leopard frog, Rana pipiens, was examined through the use of the Xenopus neural tube explant assay system (Fukuzawa and Ide, 1988). Media conditioned with pieces of skin from dorsal black spotted areas promoted melanization of neural crest cells at a significantly higher level than did media conditioned with dorsal interspot skin in the absence of extra tyrosine. All conditioned media contained exceedingly low concentrations of tyrosine. With the addition of supplemental tyrosine, the melanization capacity of conditioned media from the interspot areas was elevated to that of the spotted skin. Control media conditioned with ventral frog skin inhibited melanization, as usual, because of the presumed presence of melanization inhibiting factor (MIF). It is considered that dorsal skin contains a melanization stimulating factor (MSF) which is present in significantly higher levels in spotted skin than in interspot areas and that expression of the

particular pigmentary pattern of this leopard frog is regulated by the relative distribution of MIF, MSF, and possibly other intrinsic substances present in the skin.

- Matsunaga TO, Hruby VJ, Lebl M, Castrucci AM, Hadley ME.
Synthesis and bioactivity studies of two isosteric acyclic analogues of melanin concentrating hormone. Life Sci 51:679-685, 1992.
Abstract : Salmon melanin concentrating hormone (MCH) is a cyclic heptadecapeptide. MCH stimulates perinuclear aggregation of melanosomes within integumental melanocytes of teleost fishes resulting in skin blanching. MCH contains a disulfide bridge forming a 10-residue ring [sequence: see text]. It has been proposed that the ring is necessary for maintenance of potency. In order to test this proposal, we have synthesized two pseudo-isosteric analogues of MCH that cannot cyclize. They differed only in the polarity of the side chain group of positions 5 and 14. Serine was substituted for Cys5 and Cys14 in one peptide and L alpha-aminobutyrate (Abu) was the substitution at the two positions in the other peptide. Using a fish skin bioassay we determined that these analogues exhibit less than 1/10,000th the potency of the native hormone. These results suggest that the disulfide bridge is necessary to maintain the correct conformational and topographical features of the hormone for receptor binding and transmembrane signal transduction.

- Presse F, Hervieu G, Imaki T, Sawchenko PE, Vale W, Nahon JL.
Rat melanin-concentrating hormone messenger ribonucleic acid expression: marked changes during development and after stress and glucocorticoid stimuli. Endocrinology 131:1241-1250, 1992.
Abstract : Melanin-concentrating hormone (MCH) is a cyclic neuropeptide first isolated from fish and rats. MCH may be involved in the control of the hypothalamic-pituitary-adrenocortical axis and, more generally, of specific goal-oriented behaviors and homeostatic functions in mammals. In this paper we examine 1) the cellular distribution of MCH gene transcripts in the rat central nervous system, 2) the changes in neuronal expression of MCH mRNA during rat development, and 3) the effects of stress and hormonal stimuli on rat MCH (rMCH) gene activity. Northern blot analysis and in situ hybridization histochemistry show that mature rMCH mRNA (1.0 kilobase) is very abundant in the zona incerta and the dorsolateral hypothalamus. While this is in agreement with previous peptide mapping by immunohistochemical techniques, a surprising new result is that a few clusters of rMCH mRNA-containing cells are found outside the hypothalamus, in the olfactory tubercle and the pontine tegmentum. Developmentally, rMCH mRNA is detected on embryonic day 18; its level increases gradually during early postnatal life and rises abruptly at weaning to reach a constant value in adult rats. In addition, striking variations in rMCH mRNA length occur during postnatal development and are found to be variations in the polyadenylate tail. Interestingly, this structural modification appears to be independent of the increase in rMCH mRNA levels. The regulation of rMCH mRNA expression by glucocorticoids and chronic stress is examined by Northern blot analysis. Chronic intermittent footshock stress causes a 58% or 29% decrease in rMCH mRNA content in the whole hypothalamus after a 1- or 3-day regimen, respectively. In contrast, the rMCH mRNA level returns to normal after a 7-day regimen. Two weeks after adrenalectomy (ADX) the whole hypothalamus rMCH mRNA content decreases 2.5-fold, but rises close to the control value 3 weeks after ADX. Dexamethasone administration 2 weeks after ADX not only reverses the fall in rMCH mRNA, it even provokes a slight increase (123% of control). No change in rMCH mRNA length is observed after chronic stress or ADX and dexamethasone injection. These results provide evidence for a negative regulation of rMCH gene expression by stress and suggest a major role for glucocorticoids in a positive feedback control of rMCH gene activity.

- Svensson SP, Andersson RG, Karlsson JO.
Reciprocal changes in sensitivity to MCH and noradrenaline after denervation of teleost melanophores. Pigment Cell Res 4:252-254, 1991.
Abstract : Melanophores of isolated fish scales survive for weeks in a culture medium. During this isolation period a progressive increase in sensitivity to noradrenaline (NA) takes place. In the present study, a 100-fold increase in sensitivity to NA was found after 9 days. However, at the same time, a 12-fold decrease in sensitivity to MCH was detected.

4. Photobiology and photochemistry

- Babu V, Joshi PC.
Tryptophan as an endogenous photosensitizer to elicit harmful effects of ultraviolet B. Indian J Biochem Biophys 29:296-298, 1992.
Abstract : Owing to stratospheric ozone depletion (SOD) the natural flux of ultraviolet B (UVB) radiation (290-320 nm) is likely to increase on the earth surface. In our efforts to identify endogenous chromophores which may absorb significantly in the UVB range and subsequently induce phototoxic reactions, we have observed that tryptophan (Trp) was quite photoreactive under UVB. It enhanced considerably the oxygen-dependent photooxidation of tyrosine (Tyr) to dopachrome, a precursor of melanin. Our data suggest that UVB-sensitized Trp produces singlet oxygen (1O2) and superoxide radicals (O2-.), and these reactive forms of oxygen may contribute to membrane-, cytoplasm- and DNA-damaging effects. In the event of an increasing SOD level, other UVB chromophores may also exhibit similar phototoxic properties to lead to a definitive imbalance between cell life, injury and death.

- Bhawan J, Oh CH, Lew R, Nehal KS, Labadie RR, Tsay A, Gilchrest BA.
Histopathologic differences in the photoaging process in facial versus arm skin. Am J Dermatopathol 14:224-230, 1992.
Abstract : We used clinical criteria to study skin biopsy specimens with mild to moderate photoaging taken from the face and dorsal forearms of 74 Caucasian volunteers between the ages of 30 and 50. Facial skin had a greater number of granular cell layers, a higher degree of keratinocytic atypia, and more often showed a compact stratum corneum than arm skin. Furthermore, the dermis of facial skin had a more extensive perivascular and perifollicular lymphocytic infiltrate, more perifollicular fibrosis, a greater number of mast cells and melanophages, and thinner vascular walls than forearm skin. This study demonstrated that the photoaging process is different for face and arm skin. Appreciation of these differences should permit more refined studies of photoaging and the development of more efficient therapies.

- Chirila TV, Cooper RL, Constable IJ, Horne R.
Radiation-absorbing hydrogel-melanin blends for ocular devices. J Appl Polym Sci 44:593-604, 1992.
Abstract : Hydrophilic polymers and copolymers of 2-hydroxyethyl methacrylate, with low or high crosslinking d., are synthesized and then treated in aqueous medium with epinephrine (adrenaline) at neutral or acid pH, at room temperature, and in the presence of O and light. During this treatment, a melanin is formed and uniformly dispersed in polymers. The resulting slightly colored hydrogels display radiation-absorbing properties in the UV and visible regions of the natural spectrum. This enhances significantly their value as materials for ocular devices (contact lenses, intraocular lenses) that should protect the retina of the patients without their natural lens from potential damage induced by UV and visible (violet and blue) radiation. The incorporation of common UV absorbers leads to transmittances similar to that of the natural human lens, i.e., 30% or less at 450 nm, 40% or less at 500 nm, and no more than 50% at 700 nm. The 2-phase morphol. of the melanized hydrogels, as investigated by TEM, revealed a very fine structure comprising melanin domains of 1 to 2 nm in size. Although no proof for a network interpenetration could be provided, it is believed that the novel blends are true sequential interpenetrating polymer networks.

- Ho KK, Halliday GM, Barnetson RS.
Topical retinoic acid augments ultraviolet light-induced melanogenesis. Melanoma Res 2:41-45, 1992.
Abstract : Melanin, the natural pigment found in human skin, absorbs and protects against the ultraviolet (UV) components of sunlight. Melanin production (melanogenesis) is increased by exposure to sunlight, causing a darker skin colour which is regarded as aesthetically pleasing by many humans, who therefore expose themselves to large amounts of potentially damaging sunlight. We have found that topically applied all-trans retinoic acid, a metabolic derivative of vitamin A, greatly enhances UV light-induced melanogenesis: the same preparation on its own had no effect on skin pigmentation. An orally administered retinoid, temarotene, did not have this effect. These observations were made using a lightly pigmented mouse strain, HRA: Skh-2, and confirmed in 2

human volunteers. This is the first time that metabolic derivatives of vitamin A have been shown to augment UV light-induced melanogenesis, suggesting a role for vitamin A in this process.

- Iyengar B.
Neural differentiation as an expression of UV sensitivity of melanocytes. Acta Anat (Basel) 143:236-240, 1992.
Abstract : The present work is to study neural differentiation in melanocytes in relation to the cell cycle and UV exposure. Whole skin organ cultures of vitiliginous skin were exposed to a pulse of UV with and without prior Adriamycin treatment. It was observed that the highly dendritic marginal melanocytes are destroyed on UV exposure during the depigmentation phase but not during repigmentation. The melanocytes are resistant to UV destruction during the G2 phase as seen on Adriamycin treatment. They show a prominent increase in dendricity as well as biphasic activity to produce increased melanin and noradrenaline. Thus, the melanocytes form a UV-sensitive neural network in the skin. These responses are reminiscent of the repigmentation and depigmentation of coat color in animals exposed to extreme variations in the day/night cycles as seen at the poles.

- Jeanmougin M.
External photoprotection. Rev Prat 42:1369-1373, 1992.
Abstract : Sunscreens go beyond the field of cosmetology to enter that of preventive or curative medicine. The anti-sun product is a preparation in which "filters" selective for UVB and/or UVA are associated with mineral powders called "screens" that are either dissolved or dispersed in an excipient. Modern products also contain compounds protecting against free radicals, anti-inflammatory agents, biosynthetic melanin or topical psoralens. Sunscreen efficacy tests provide "protection factors" against UVA and/or UVB, and/or infrared radiations. Testing the resistance of these indices to time and to immersion or sudation is necessary to evaluate the substantiality and remanence of sunscreens. These parameters enable sunscreens to be classified into four families with increasing activities: weak, fair, high and major protection. Protecting healthy subjects against solar radiation requires "common sense" and the choice of a sunscreen based on its dermatological risk depending on the phototype, the circumstances of exposure to sun, the regions exposed and the chemical formula of the compound.

- Kurban AK, Morrison PR, Trainor SW, Tan OT.
Pulse duration effects on cutaneous pigment. Lasers Surg Med 12:282-287, 1992.
Abstract : Melanin, an endogenous chromophore in pigment containing cells in skin, is being specifically altered by lasers using the principle of selective photothermolysis (SPT). This implies that a combination of specific laser parameters of wavelength, pulse duration, spotsize, and energy density are required to confine the delivered laser energy to the targeted cells alone. Because the bulk of cutaneous pigment is localized to epidermal basal cells, pigmentary incontinence has been found to occur in skin exposed to laser irradiation. This study demonstrates that pulse duration or exposure time of the laser affect the severity of pigmentary incontinence induced. Pigment granules are more abundant, aggregated, and located deeper in the dermis following exposure to 500 nsec pulse duration than 100 nsec at a wavelength of 504 nm. This relationship appears to be independent of the laser energy density used.

- Osorio D, Bossomaier TR.
Human cone-pigment spectral sensitivities and the reflectances of natural surfaces. Biol Cybern 67:217-222, 1992.
Abstract : The evolution of visual pigment spectral sensitivities is probably influenced by the reflectance spectra of surfaces in the animal's environment. These reflectances, we conjecture, fall into three main classes: i. Most inorganic and many organic surfaces, including tree bark, dead leaves and animal melanin pigmentation, whose reflectance increases gradually as a function of wavelength. ii. Living leaves, which contain chlorophyll, have a sharp reflectance peak at about 555 nm. iii. Flowers, fruit and other signaling colours that have co-evolved with animal vision typically do not reflect strongly at the same wavelength as leaves, and present a colour contrast against a leafy background. These three spectral functions we call 'grey-red', 'leaf-green' and 'leaf-contrast'

respectively. This simple categorisation allows us to interpret the spectral tuning of human cone pigments in a way that might not seem possible given the wide variety of colours present in nature. In particular L-(red) cones will capture the highest possible proportion of photons reflected by leaves, and M-(green) cones will capture about 10% fewer photons both from leaves and from 'grey-red' surfaces. These observations have some clear implications for our understanding of the evolution of trichomacy and the trade-off between chromatic and luminance vision in Old-World Primates.

- Rapaport DH, Herman KG, LaVail MM.
Epi-polarization and incident light microscopy readily resolve an autoradiographic or heavy metal label from an obscuring background or second label. J Neurosci Methods 41:231-238, 1992.
Abstract : Difficulty encountered in resolving grains of exposed photographic emulsion in autoradiographs of the densely melanized retinal pigment epithelium was solved by using epi-polarized or incident light microscopy. The apparatus used included a metallurgical illuminator specifically designed for epi-polarization microscopy or, as a less expensive but only slightly less effective alternative, a modified fluorescence illuminator. The black melanin granules absorb incident light (as they do in vivo) while the silver grains reflect it producing a "darkfield-like" representation. Brightfield and darkfield-like images can be alternated easily and quickly, or both can be viewed simultaneously. Epi-polarization microscopy has wider application in resolving a reflective label over any opaque background staining or dark second label.

5. Neuromelanins

- Bannon MJ, Poosch MS, Xia Y, Goebel DJ, Cassin B, Kapatos G.
Dopamine transporter mRNA content in human substantia nigra decreases precipitously with age. Proc Natl Acad Sci USA 89:7095-7099, 1992.
Abstract : The dopamine transporter is the primary means of inactivating synaptic dopamine as well as a major site of action for psychostimulants (such as cocaine and amphetamine) and for neurotoxins that induce parkinsonism. In the present study, a human dopamine transporter partial cDNA clone obtained by polymerase chain reaction exhibited 87% and 89% identity at the nucleic acid and amino acid levels, respectively, with transmembrane domains 3-5 of the rat homolog. This clone was used to quantitate human dopamine transporter mRNA by nuclease protection assay. The postmortem content of dopamine transporter mRNA in the substantia nigrae of 18- to 57-yr-old subjects was relatively constant, while in subjects greater than 57 yr old, a precipitous (greater than 95%) decline in substantia nigra dopamine transporter mRNA was evident. In contrast, tyrosine hydroxylase mRNA in the same samples declined in a linear manner with increasing age. In situ hybridization experiments confirmed the profound loss of dopamine transporter gene expression in melanin-positive (presumptive dopamine) nigral neurons. These data may begin to shed light on compensatory changes occurring in human dopamine neurons during normal aging.

- Ben-Shachar D, Eshel G, Riederer P, Youdim MB.
Role of iron and iron chelation in dopaminergic-induced neurodegeneration: implication for Parkinson's disease. Ann Neurol; 32 Suppl pS105-10, 1992.
Abstract : Recent studies in Parkinson's disease suggest that the degeneration of the nigrostriatal melanin-containing dopaminergic neurons results from toxic effects of free radicals, which are generated during dopamine metabolism in the substantia nigra (SN). This has been linked to the selective accumulation of iron, a known catalyst of radical formation, in the zona compacta of the SN. We have shown that interaction of iron with melanin may result in a high affinity binding of iron to melanin (KD = 13.0 +/- 0.15 nM). Indeed, x-ray analysis of melanized dopamine neurons of parkinsonian SN has shown an interaction of iron with melanin that is absent in control brains. In the presence of excess Fe3+, melanin potentiates iron-induced lipid peroxidation. Since iron chelators prevent lipid peroxidation, we have ascertained the ability of the iron chelator deferoxamine to prevent the lesion of the nigrostriatal dopamine neuron induced by 6-hydroxy dopamine (6-OHDA). Our results demonstrated that intraventricular injection of 130 ng deferoxamine to rats prior to 250

micrograms of 6-OHDA partially prevented the decrease in striatal dopamine content caused by 6-OHDA (56% reduction vs 90%, respectively). This protection was sufficient to produce normal dopamine-related behavioral responses. These results suggest that iron and iron chelators play a crucial role in the process of dopaminergic neurodegeneration and neuroprotection. The latter is further supported by our recent findings that intranigral injection of iron (50 micrograms) resulted in a substantial selective decrease of striatal dopamine (95%) and impaired dopamine-related responses.

- Gibb WRG.
Melanin, tyrosine hydroxylase, calbindin and substance P in the human midbrain and substantia nigra in relation to nigrostriatal projections and differential neuronal susceptibility in Parkinson's disease. Brain Res 581:283-291, 1992.
Abstract : The anatomy of melanin-containing neurons and other midbrain structures was examined by tyrosine hydroxylase (TH), calbindin D28k, and substance P immunostaining. Greater than 95% of cells in the substantia nigra pars compacta contained melanin, but densely packed cells in a ventral tier had a low content of melanin and loosely packed cells in a dorsal tier had a high content of melanin. Approx. 60% in the gamma group and 40% in the retrorubral nucleus had a low content of melanin. TH immunostaining was moderate in both the ventral and dorsal tiers, but more intense in the gamma group and retrorubral nucleus. Calbindin D28k was absent from the ventral and dorsal tiers, but present in the gamma group and retrorubral nucleus. The distribution of substance P was similar to that of calbindin D28k. In the light of primate tracing studies these findings suggest that the ventral tier of the pars compacta projects to striosomes of the striatum, and the dorsal tier, gamma group and retrorubral nucleus to the matrix compartment. The ventral tier is more vulnerable than the dorsal tier in Parkinson's disease, but the cells contain less melanin. Neither tier contains calbindin D28k. This differential vulnerability between the ventral and dorsal tiers cannot be explained by melanin or calbindin D28k.

- Hirsch EC.
Why are nigral catecholaminergic neurons more vulnerable than other cells in Parkinson's disease? Ann Neurol 32(Suppl):S88-S93, 1992.
Abstract : Although the cause of neuronal death in Parkinson's disease remains unknown, a hyperoxidation phenomenon has been implicated as a potential cytotoxic mechanism. Catecholaminergic neurons containing neuromelanin, an autoxidation byproduct of catecholamines, are more vulnerable in Parkinson's disease than nonmelanized catecholaminergic neurons. High levels of CuZn superoxide dismutase mRNA have been observed in the substantia nigra, suggesting that high levels of oxygen free radicals are indeed produced in the structure. Catecholaminergic neurons surrounded by a low density of glutathione peroxidase cells are more susceptible to degeneration in Parkinson's disease than those well protected against oxidative stress. The nigral content in iron, a compound that exacerbates the production of free radicals in catecholaminergic neurons, is increased in Parkinson's disease. Altogether these data suggest that hyperoxidation may participate in the selective vulnerability of catecholaminergic neurons in Parkinson's disease.

- Hornykiewicz O.
Mechanisms of neuronal loss in Parkinson's disease: a neuroanatomical-biochemical perspective. Clin Neurol Neurosurg 94 (Suppl):S9-S11, 1992.
Abstract : It is shown that the specific inter- and subregional patterns of striatal dopamine loss in idiopathic Parkinson's disease can serve as a criterion for the evaluation of neurotoxic processes suggested to play an etiological role in this disorder. Based on this premise, the possibility is examined that dopamine-based free radicals (oxidative stress), or a MPTP-like mechanism may be the primary cause of the substantia nigra cell death in idiopathic Parkinson's disease. It is concluded that the most likely determinant of the specific patterns of nigral cell loss and striatal dopamine deficit might be the peculiar topomorphological arrangement of the melanin-containing neurones in the human substantia nigra.

- Jellinger K, Kienzl E, Rumpelmair G, Riederer P, Stachelberger H, Ben-Shachar D, Youdim MB. **Iron-melanin complex in substantia nigra of parkinsonian brains: an x-ray microanalysis.** J Neurochem 59:1168-1171, 1992.
  Abstract : Using energy-dispersive x-ray analysis on an electron microscope working in the scanning transmission electron microscopy mode equipped with a microanalysis system, we studied the subcellular distribution of trace elements in neuromelanin-containing neurons of the substantia nigra zona compacta (SNZC) of three cases of idiopathic Parkinson's disease (PD) [one with Alzheimer's disease (AD)] and of three controls, in Lewy bodies of SNZC, and in synthetic dopamine-melanin chemically charged or uncharged with Fe. Weak but significant Fe peaks similar to those of a synthetic melanin-Fe3+ complex were seen only in intraneuronal highly electron-dense neuromelanin granules of SNZC cells of PD brains, with the highest levels in a case of PD plus AD, whereas a synthetic melanin-Fe2+ complex showed much lower iron peaks, indicating that neuromelanin has higher affinity for Fe3+ than for Fe2+. No detectable Fe was seen in nonmelanized cytoplasm of SNZC neurons and in the adjacent neuropil in both PD and controls, in Lewy bodies in SNZC neurons in PD, and in synthetic dopamine-melanin uncharged with iron. These findings, demonstrating for the first time a neuromelanin-iron complex in dopaminergic SNZC neurons in PD, support the assumption that an iron-melanin interaction contributes significantly to dopaminergic neurodegeneration in PD and PD plus AD.

- Kastner A, Hirsch EC, Lejeune O, Javoy-Agid F, Rascol O, Agid Y. **Is the vulnerability of neurons in the substantia nigra of patients with Parkinson's disease related to their neuromelanin content?** J Neurochem 59:1080-1089, 1992.
  Abstract : The contribution of neuromelanin (NM) to the pathogenesis of Parkinson's disease (PD) has long been suspected. In particular, a correlation has been reported between the estimated cell loss in the mesencephalic dopaminergic cell groups and the percentage of NM-pigmented neurons in these cell groups. To test whether the amount of pigment per cell is a critical factor or whether the presence of NM within a neuron is sufficient to account for the degeneration of dopaminergic neurons, the NM content was measured in each neuron from representative sections throughout the ventral mesencephalon of four controls subjects and four patients with PD. Intraneuronal NM was quantified by a densitometric method, using known amounts of synthetic melanin as standards. In control brains, the distribution of melanized neurons in the nigral complex showed a high proportion of lightly melanized neurons in the ventral tegmental area and the pars alpha and gamma of the substantia nigra (SN), whereas heavily melanized neurons were mostly located in the pars beta and lateralis of the SN. An inverse relationship was observed between the percentage of surviving neurons in PD compared with controls and the amount of NM they contain, suggesting that the vulnerability of the dopaminergic neurons is related to their NM content. Factors other than NM may be involved in the differential vulnerability of catecholaminergic neurons in PD. In particular, the constant topography of the cell loss suggests that cell position within the nigral complex is a key factor.

- Kawaguchi K. **Susceptibility of organ of Corti with or without melanin to acoustic overstimulation.** Nippon Jibiinkoka Gakkai Kaiho 95:556-566, 1992.
  Abstract : Albino and pigmented guinea pigs were compared in terms of susceptibility to acoustic trauma. The animals were exposed to a 4 kHz pure tone of 120 dB for 60 min. N1 thresholds of CAP were measured before and after the acoustic exposure. Changes in the outer hair cell and stria vascularis were studied using SEM and TEM. After acoustic trauma, N1 thresholds were more elevated in the albino than in the pigmented guinea pigs. Also, pathological changes in the outer hair cell and stria vascularis were more severe in the albino animals. A noteworthy finding in the stria vascularis was that the melanin in intermediate cells had moved into marginal cells. This melanin migration may be possibly involved in mechanisms underlying prevention of acoustic trauma.

- Knoll J, Toth V, Kummert M, Sugar J. **(-)deprenyl and (-)parafluorodeprenyl-treatment prevents age-related pigment changes in the substantia nigra. A TV-image analysis of neuromelanin.** Mech Ageing Dev 63:157-163, 1992.
  Abstract : With the aid of TV-image analysis the number, the area and the density features of melanin

granules in neurocytes of the substantia nigra in a group of 3-month-old naive male rats and in a 21-month-old group of male rats treated for 18 months with saline, (-)deprenyl and (-)parafluorodeprenyl, respectively, were determined. According to the Kolmogorov 2-sample test the two drug-treated groups do not differ significantly from each other in the number, total area and area of one pigment granule. In the aged saline-treated group the number of melanin granules decreased significantly and the area of the melanin granules increased in comparison to young controls as well as to the drug-treated groups.

- Swartz HM, Sarna T, Zecca L.
Modulation by neuromelanin of the availability and reactivity of metal ions. Ann Neurol 32 (Suppl):S69-S75, 1992.
Abstract : Presence of neuromelanin is likely to alter significantly the amount, distribution, reactivity, and consequences of reactivity of metal ions in those parts of the brain that contain neuromelanin. The effects are complex and can be predicted only with detailed knowledge of the system because (1) melanins are strong binders of metal ions and many organic molecules; (2) the effect of binding, depending on the circumstances, can increase or decrease reactivity of metal ions; and (3) melanins can generate and react with oxidation-reduction-active species such as hydrogen peroxide, hydroxyl radicals, superoxide anions, and singlet oxygen. Neuromelanin has some significant differences from most other natural melanins because of its mode of formation (by autooxidation rather than enzymatically) and its composition (it probably is a copolymer derived from dopamine and glutathione). It probably is not possible to understand fully the role of metal ions in oxidative damage in the brain without having an adequate understanding of the structure and reactivity of neuromelanin.

- Toth V, Kummert M, Sugar J, Knoll J.
A procedure for measuring neuromelanin in neurocytes by a TV-image analyser. Mech Ageing Dev 63:215-221, 1992.
Abstract : With the aid of a Robotron A6471 type TV-image analyser the number, total area, area of one granule and density features (sum, average of gray values and average gray value of one pigment granule) of melanin granules in neurocytes of the substantia nigra in 3-month-old and 3-year-old male rats were determined. The number of cells in sections of identical areas was similar in the young and old rats. A statistically non-significant difference between the two age cohorts in the proportion of neurocytes with and without melanin was found; 773 (48.1%) and 853 (51.8%), in the young rats and 1219 (65.1%) and 652 (34.8%) in the old ones. Within the melanin containing neurocytes, however, statistically conspicuous, age-related differences in the number, area and density features of melanin granules were discovered. The majority of the neurocytes in young rats contained numerous, small sized neuromelanin granules, whereas in the majority of the neurocytes of old rats smaller numbers of large sized, neuromelanin granules were detected.

6. Genetics, molecular biology

- Lowings P, Yavuzer U, Goding CR.
Positive and negative elements regulate a melanocyte-specific promoter. Mol Cell Biol 12:3653-3662, 1992.
Abstract : Melanocytes are specialized cells residing in the hair follicles, the eye, and the basal layer of the human epidermis whose primary function is the production of the pigment melanin, giving rise to skin, hair, and eye color. Melanogenesis, a process unique to melanocytes that involves the processing of tyrosine by a number of melanocyte-specific enzymes, including tyrosinase and tyrosinase-related protein 1 (TRP-1), occurs only after differentiation from the melanocyte precursor, the melanoblast. In humans, melanogenesis is inducible by UV irradiation, with melanin being transferred from the melanocyte in the epidermis to the surrounding keratinocytes as protection from UV-induced damage. Excessive exposure to UV, however, is the primary cause of malignant melanoma, an increasingly common and highly aggressive disease. As an initial approach to

understanding the regulation of melanocyte differentiation and melanocyte-specific transcription, we have isolated the gene encoding TRP-1 and examined the cis- and trans-acting factors required for cell-type-specific expression. We find that the TRP-1 promoter comprises both positive and negative regulatory elements which confer efficient expression in a TRP-1-expressing, pigmented melanoma cell line but not in NIH 3T3 or JEG3 cells and that a minimal promoter extending between -44 and +107 is sufficient for cell-type-specific expression. Assays for DNA-protein interactions coupled with extensive mutagenesis identified three factors, whose binding correlated with the function of two positive and one negative regulatory element. One of these factors, termed M-box-binding factor 1, binds to an 11-bp motif, the M box, which acts as a positive regulatory element both in TRP-1-expressing and -nonexpressing cell lines, despite being entirely conserved between the melanocyte-specific tyrosinase and TRP-1 promoters. The possible mechanisms underlying melanocyte-specific gene expression are discussed.

- Matsunaga J, Takeda A, Tomita Y, Hara M, Shibahara S, Tagami H.
Cloning and sequence analysis of the tyrosinase gene from a patient with tyrosinase-positive oculocutaneous albinism. J Dermatol Sci 3:181-185, 1992.
Abstract : Tyrosinase is synthesized on membrane-bound ribosomes and transported into melanosomes through smooth endoplasmic reticulum and Golgi apparatus. Melanin polymers are produced only in melanosomes but never in smooth endoplasmic reticulum or Golgi apparatus, indicating that posttranslational modifications of tyrosinase are completed with melanosomes where tyrosinase becomes an active form. Based on a working hypothesis that tyrosinase-positive oculocutaneous albinism is a consequence of the structurally altered tyrosinase due to a point mutation in the gene of its gene coding for a glycosylation site or a membrane-binding site, which leads to the impairment in the posttranslational modification of tyrosinase and its catalytic activity, we have cloned the tyrosinase gene of one patient affected with tyrosinase-positive oculocutaneous albinism and determined its nucleotide sequence. Thus demonstrated all exons' nucleotide sequence of the patient's tyrosinase gene was found to be identical to that of the wild-type gene. The results indicate that the patient's tyrosinase itself is not altered. We therefore propose that the molecular basis for the development of tyrosinase-positive oculocutaneous albinism exists as a defect in other proteins required for the activation of tyrosinase or in other regions of the tyrosinase gene.

- Rose NC, Menacker SJ, Schnur RE, Jackson L, McDonald-McGinn DM, Stump T, Emanuel BS, Zackai EH.
Ocular albinism in a male with del (6)(q13-q15): candidate region for autosomal recessive ocular albinism? Am J Med Genet 42:700-705, 1992.
Abstract : We describe a boy with an interstitial deletion of 6(q13-q15) and include "coarse" facial features, upslanting palpebral fissures, thin vermilion border of the upper lip, elongated philtrum, developmental delay, and profound hypotonia. The child's eye findings, pedigree, paucity of maternal ocular changes, and lack of melanin macroglobules in the skin suggest that this individual's phenotype is clinically similar to that of autosomal recessive ocular albinism. Though it is possible that this deletion and his ophthalmic disorder are coincidental, we postulate that the ocular albinism may be due to hemizygosity for a paternally derived ocular albinism gene located on chromosome 6 in the region q13-q15. This patient's deletion is secondary to a recombination of a maternal intrachromosomal inverted insertion of this region. Of the 7 reported 6q1 deletions, this is the only case that is due to a familial chromosome rearrangement.

- Shibata K, Muraosa Y, Tomita Y, Tagami H, Shibahara S.
Identification of a cis-acting element that enhances the pigment cell-specific expression of the human tyrosinase gene. J Biol Chem 267:20584-20588, 1992.
Abstract : To identify the cis-acting element that is responsible for the pigment cell-specific expression of the human tyrosinase gene, the authors analyzed the promoter activity of its 5'-flanking region by transient expression assays. The fusion genes were constructed by inserting the 5'-flanking region of the human tyrosinase gene upstream from the firefly luciferase gene and were introduced into human melanoma cells and HeLa cells. The element was located between 2.7 and 1.8 kb upstream from the transcription initiation site, and enhanced the transient expression of the luciferase reporter gene in

melanoma cells, but not in HeLa cells, the tyrosinase gene expression of which is not detectable. Using the fusion genes containing putative enhancer elements under the control of the heterologous SV40 promoter, the pigment cell-specific enhancer of .apprx.200 bp was located between -2.0 and -1.8 kb and the core sequence was a 39-bp region. This 39-bp core element was then confirmed to direct the melanoma cell-specific expression of the reporter gene under the tyrosinase gene promoter. The authors propose that this core element is responsible for the pigment cell-specific expression of the human tyrosinase gene.

- Tripathi RK, Strunk KM, Giebel LB, Weleber RG, Spritz RA.
Tyrosinase gene mutations in type I (tyrosinase-deficient) oculocutaneous albinism define two clusters of missense substitutions. Am J Med Genet 43:865-871, 1992.
Abstract : Type I (tyrosinase-deficient) oculocutaneous albinism (OCA) results from mutations of the gene encoding tyrosinase, the enzyme that catalyzes the first 2 steps of melanin pigment biosynthesis. In type IA (tyrosinase-negative) OCA tyrosinase enzymatic activity is completely absent, and in type IB ("yellow") OCA tyrosinase activity is greatly reduced. Here, we describe 11 novel mutations of the tyrosinase gene in Caucasian patients with these 2 forms of type I OCA. Type I OCA in Caucasians appears to result from a great variety of different uncommon alleles. More than 80% of the known missense substitutions associated with type I OCA cluster within 2 relatively small regions of the tyrosinase polypeptide, suggesting that these may correspond to functionally important sites within the enzyme.

- Uchihi R, Tamaki K, Kojima T, Yamamoto T, Katsumata Y.
Deoxyribonucleic acid (DNA) typing of human leukocyte antigen (HLA)-DQA1 from single hairs in Japanese. J Forensic Sci 37:853-859, 1992.
Abstract : The deoxyribonucleic acid (DNA) typing of human leukocyte antigen (HLA)-DQA1 from single hairs is described. HLA-DQA1 genotypes could be determined from single plucked hair roots. However, it was not easy to type HLA-DQA1 with hair shaft portions. Increase in the specimens of hair shaft portions (over 10 cm in length) to get sufficient DNA caused inhibition of polymerase chain reaction (PCR). Synthetic melanin as well as the one extracted from hairs inhibited the PCR of the genomic DNA template when added to the PCR reaction at the concentrations over than 15 ng/100 microL. Therefore, typability of hair shaft portions seems to depend on the delicate balance of the concentrations of DNA and the contaminated melanin in the final DNA extracts.

7. Tyrosinase, TRP1, TRP2 and other enzymes

- Chen LY, Leu WM, Wang KT, Lee YHW.
Copper transfer and activation of the Streptomyces apotyrosinase are mediated through a complex formation between apotyrosinase and its trans-activator MelC1. J Biol Chem 267:20100-20107, 1992.
Abstract : The melanin operon (melC) of Streptomycetes antibioticus is composed of two genes that encode MelC1 and MelC2 proteins. MelC1 has been suggested as a trans-activator which can facilitate the incorporation of copper into the apotyrosinase (MelC2) (Lee YHW et al, 1988). However, the mol. mechanism of the trans-activation or copper-transfer process mediated through MelC1 to MelC2 is not clear yet. In this study, apotyrosinase was found in both the extracellular fraction and cell ext. from cells grown in copper-deficient medium. Gel-filtration and immunoaffinity chromatogs. demonstrated that apotyrosinase (MelC2) formed a stable complex with MelC1 in the intra- and extracellular fractions. Furthermore, addition of copper ion to the complex generated tyrosinase activity. The MelC1-MelC2 complex was purified to near homogeneity by DE52 and phenyl-agarose chromatogs. In conjunction with fast-protein liquid gel filtration chromatography and NH2-terminal sequencing analysis, the results indicated that the stoichiometric ratio of MelC1 and MelC2 in the purified complex was 1:1. Essentially no copper was found in the complex. Addition of copper ion to the purified complex resulted in incorporation of approx. 2 mols. of copper ion and the mature active tyrosinase was gradually released from the complex. Taken together, these results demonstrate that the mol. mechanism of activation of Streptomyces apotyrosinase by its trans-activator MelC1 is

initially mediated via a binary complex formation between these two proteins, followed by incorporation of copper ion. This activation mechanism accounts for the essential role of MelC1 in the expression of melanin operon.

- Leu WM, Chen LY, Liaw LL, Lee YHW.
Secretion of the Streptomyces tyrosinase is mediated through its trans-activator protein, MelC1. J Biol Chem 267:20108-20113, 1992.
Abstract : The tyrosinase of Streptomyces antibioticus is encoded by the 2nd open reading frame, melC2, of the melanin operon (melC). The upstream open reading frame melC1 specifies a 146-amino acid protein with a typical NH2-terminal signal peptide characteristic of a secretory protein. The MelC1 protein is involved in the transfer of copper ion to apotyrosinase MelC2 via binary complex formation. To investigate whether the export of tyrosinase is also dependent on MelC1, a mutational study of its signal peptide sequence was performed. Four different mutants were obtained. Mutation at the pos. charged region (mutant M-6LE, Arg6-Arg7.fwdarw.Leu6-Glu7) or the hydrophobic region (mutant M-16D, Val16.fwdarw.Asp16) led to Mel- phenotypes. These lesions caused a severe 7-10-reduction of the export of both the MelC1 and MelC2 proteins and a concomitant accumulation of the 2 proteins in the cytosolic fraction. The cell-associated tyrosinase activity in M-6LE but not in the M-16D mutant was dramatically reduced to 4% of the activity found in the wild type strain, suggesting that the basic NH2 terminus of MelC1 is also important for the trans-activation function of this protein. Nevertheless, the defects on the trans-activation and/or secretory functions of MelC1 in mutants M-6LE and M-16D are not due to the impairment of the formation of the MelC1.MelC2 complex. The translation of melanin operon genes in these two mutants also decreased. In contrast, the tyrosinase activity and the secretion of MelC2 were not affected if the mutations occurred at the putative cleavage site of the signal peptidase (e.g. mutant M-29SM, Arg29-Ala30.fwdarw.Ser29-Met30 or mutant 29-SMG, Arg29-Ala30-Asp31.fwdarw.Ser29-Met30-Gly31). Addnl., tyrosinase activity and its export were abolished in a MelC1-neg. mutant, M-950. Taken together, these results demonstrate that a functional MelC1 is essential for tyrosinase secretion and activity. Furthermore, the results suggest that, like other secretory proteins, basic and hydrophobic residues in the MelC1 signal sequence are an important feature of the signal peptide and play a pivotal role in the secretion of both the MelC1 and MelC2 proteins. These results also establish a novel role for MelC1 on tyrosinase transport in addition to a role of copper transfer to tyrosinase.

- Watanabe K, Araki M, Iwasaki H.
The embryonic pineal body as a multipotent organ. Microsc Res Tech 21:218-226, 1992.
Abstract : The repertoire of differentiating potency of mammalian and avian pineal cells has been examined utilizing cell culture technique. Skeletal muscle fibers are differentiated from pineal cells of the rat under the usual culture condition and from those of quail under hypertonic conditions. Myogenesis of pineal cells may be explained from the ontogeny of the pineal body. Anlagen of a pineal body are situated in bilateral cephalic neural folds, which also supply multipotent neural crest cells. In some conditions, almost all quail pineal cells are able to differentiate into pigmented epithelial cells and/or lens cells. Opsin containing cells found in culture of rat pineal cells may be in a similar category reflecting the "third eye": the phylogenetic ancestor of the pineal body of avian and mammalian species. Neuron-like cells have also been reported and neuronal morphology has been intensified under the effect of testicular hyaluronidase. The cytodifferentiation described above is suggested to be different expressions of a single type of progenitor cells in the pineal body. In relation to multipotentiality of pineal cells, the original differentiating state of pineal cells is interesting; it has been found that tyrosinase is expressed from the beginning of pineal formation and that its expression is stage-specific (during embryonic period) and site-specific (predominance in the dorsal half of the pineal body and in the apical cytoplasm of the pineal cell). In the 8 day quail embryo used for culture studies, three differentiating states as to tyrosinase are noticed. However, the distinction may be apparent, as even the cells negative in tyrosinase in this stage are still ready to express tyrosinase in the suitable culture condition.

8. Melanoma and other pigmented tumours

- Aoki O, Kono M, Mishima Y.
MR (magnetic resonance) imaging and pathologic correlation on hamster malignant melanoma and clinical application. Kyoto Daigaku Genshiro Jikkensho, Tech. Rep., KURRI-TR-357, 167-182, 1991.
Abstract : To increase the diagnostic accuracy of magnetic resonance imaging (MRI) of human malignant melanoma, MR images of hamster malignant melanoma, which resemble human malignant melanoma biol. and pathol., were obtained and compared with the pathological findings of the tumor. The viable part of the tumor was demonstrated as slightly hyperintense area on the T1-weighted images and hyperintense area on the T2 images. The coagulation necrosis was hyperintense on the T1 images and slightly hyperintense on the T2 images. The liquefaction necrosis was quite hypointense on the T1 images and quite hyperintense on the T2 images. Consequently, MRI could distinguish the viable tumor from the necrotic part of hamster melanoma on the T1 and the T2 images. Based on this experimental study, MRI on 24 lesions of human malignant melanoma was performed. In 21 out of 24 lesions, the viable part of the melanoma was demonstrated as slightly hyperintense on the T1 images and hyperintense on the T2 images. Only 3 lesions were hyperintense on the T1 images and hypointense on the T2 images due to the paramagnetic effect of melanin. It was thought that the signal intensity of the melanoma was more affected by necrotic changes in the tumor than the paramagnetic effect of melanin.

- Aubert C, Ali-Mehidi S, Rouge F, Voulot C.
Differentiation of new metastatic variants of B16 melanoma under different culture conditions. Pigment Cell Res 5:12-24, 1992.
Abstract : The purpose of this study was to examine the differentiation of variant tumors of the B16 metastatic melanoma when tumors were grown serially under different culture conditions and transplanted into C57BL/6J black mice, lethal yellow Ay/a, albino c/c, and C+/c mutant mice. Morphological and biochemical markers of melanogenesis were examined in cells in culture and in the corresponding tumors. Cellular pigmentation was assessed in terms of the levels of DOPA and 5-S-CD and in terms of tyrosinase activity in the various cell lines and tumors. The observed change from high to low metastatic capacity, which was dependent on culture conditions, appeared to be unrelated to melanogenesis even though changes were observed in the biochemical melanotic phenotype. Overall, tumor cells from spontaneous pulmonary metastases appear to differentiate in ways that are unrelated to the instability of experimental metastatic capacity. The melanotic phenotype in albino c/c and C+/c mice was dependent on the phenotype of the parental tumors. A marked difference was observed between two pigmentation compartments, one of which was stable in the B16 control, while the other was unstable in YB16 and MB16 variant cells and in the tumors derived from them. It appears, therefore, that the metastatic capacity of B16 metastatic variants is changeable and is independent of the unstable melanogenic behavior. The production of metastases and the differentiation of tumors in the present experiments appeared to be related to the genetic background of the mice and the epigenetic metabolic environment of tumors and cells.

- Bloom PA, Ferris JD, Laidlaw DA, Goddard PR.
Magnetic resonance imaging. Diverse appearances of uveal malignant melanomas. Arch Ophthalmol 110:1105-1111, 1992.
Abstract : Fifteen patients with uveal malignant melanomas were studied by magnetic resonance imaging. The magnetic resonance imaging appearances varied from those that have been reported previously to be characteristic of these tumors. In our series, malignant melanomas were of high signal on the T1 sequence and of variable but usually also of high signal on the T2 and Short Tau Inversion Recovery (STIR) sequences, a signal combination rarely described before. We postulate that magnetic resonance imaging appearances may be dependent on variations in histologic factors and on the type and field strength of the scanner used. It is widely believed that the paramagnetic melanin in malignant melanomas gives these tumors characteristic magnetic resonance imaging appearances, but our finding of diverse magnetic resonance imaging appearances for proved malignant melanomas suggests that this may not always be the case. We advise caution in diagnosing malignant melanomas from magnetic resonance imaging appearances alone.

- Cope R, Debou JM.
Malignant melanoma of the anal canal. Apropos of 8 patients and review of the literature. Chirurgie 117:431-436, 1991.
Abstract : The authors present 8 patients with malignant melanoma of the anal canal. By including these patients with those already reported in the literature, they systematically analyse the various aspects of this exceptional site. These tumors always develop at the pectinate line and transitional mucosa, while rectal localizations corresponds to contiguous extension from a melanoma of the anal canal. The clinical features are only suggestive when the lesions appear pigmented macroscopically. The histological certainty depends of the demonstration of melanin pigment and is simple when the tumor is pigmented (75% of cases). The diagnostic is more complex in the case of nonpigmented tumors, but is facilitated by electronic microscopy and certain immune markers (Proteins S 100). The constantly very severe prognosis depends of the depth of invasion, frequently assessed by Breslow's method, and lymph node invasion and metastatic spread are very frequent (88% of patients) either at the time of diagnosis or later in the course of the disease. The mean survival of 24 months does not appear to be influenced by treatment which is always surgical, either local excision of abdomino-perineal resection. Abdomino-perineal resection may be preferred in the case of a very small tumor less than 2 mm thick in the absence of any visceral metastases. Radiotherapy to extensive tumors is only palliative treatment and chemotherapy has not been found to be effective to date.

- Fournie JW, Hawkins WE, Walker WW.
Adenocarcinoma of the retinal pigment epithelium in the guppy Poecilia reticulata Peters. J Comp Pathol 106:429-434, 1992.
Abstract : A single case of adenocarcinoma of the retinal pigment epithelium occurred in a guppy, Poecilia reticulata, Peters. This is the first such tumour reported from fishes. The left eye of the affected fish was severely exophthalmic because of a large intraocular tumour mass. The tumour, which displaced normal retina anteriorly, consisted mainly of melanin-containing epithelial cells. Neoplastic cells were bilayered and arranged in a tubular pattern. The tumour was confined to the orbit. Although the specimen was from a group exposed to a mixture of halogenated organic compounds, the lesion was not considered to have been chemically induced because of its rare occurrence within the group as a whole.

- Gruber JR, Ohno S, Niles RM.
Increased expression of protein kinase C alpha plays a key role in retinoic acid-induced melanoma differentiation. J Biol Chem 267:13356-13360, 1992.
Abstract : Differentiation of B16 mouse melanoma cells induced by retinoic acid (RA) is preceded by a large increase in protein kinase C alpha (PKC alpha) mRNA and protein. To determine the role of PKC alpha in the differentiation program, we stably transfected B16-F1 cells with a plasmid containing the full length PKC alpha cDNA driven by an SV40 promoter. Two out of thirty-two colonies screened were determined to overexpress PKC by 2-4-fold according to Western blot analysis and PKC enzyme activity. When compared to control cells (wild-type cells and cells transfected only with the neomycin resistance gene), PKC alpha overexpressing clones displayed longer doubling times, diminished anchorage-independent growth, and increased melanin production. RA treatment of control cells mimicked these phenotypic characteristics. When injected subcutaneously into syngeneic mice, PKC alpha overexpressing clones produced smaller tumors and had longer latencies than control cells. These findings, combined with the fact that phorbol esters down-regulate PKC and antagonize RA action suggest that PKC alpha plays a key role in the RA-induced melanoma differentiation.

- Jakic-Razumovic J, Cacic-Pilipovic M, Cuzic S.
Histologic criteria for the diagnosis of superficially spreading melanoma in patients with proven metastases. Acta Med Croatica 45:325-333, 1991.
Abstract : The majority of authors agree that the prognosis of malignant melanoma depends on the depth of extension of malignant melanocytes into the dermis. A number of studies were performed to determine histologic characteristics of primary malignant melanoma that already metastasized. Their goal was to predict the biological behaviour of malignant melanoma by the histological picture.

The study was designed to establish histological characteristics of superficial spreading melanoma. To prove the malignant biological behaviour, only superficial spreading melanomas with metastases in lymph nodes were analysed. The aim of the study was to see whether the melanoma's biological behaviour could be predicted on the basis of its histological picture. A total of 39 cases of superficially spreading malignant melanoma from the Department of Pathology, University Hospital Center Rebro, Zagreb, were analysed. The sections were taken from 5 different places, fixed in 10% purified formalin, paraffin embedded and stained with hematoxylin-eosin. In some cases the sections were additionally stained with Fontana to prove melanin. The sections were analysed by light microscopy. The following histological characteristics were analysed: 1. poor circumscription of the intraepidermal melanocytes; 2. individual melanocytes extending laterally; 3. number of atypical melanocytes above the basal membrane; 4. marked variation in the size and shape of melanocytic nests; 5. tendency of melanocytic nests towards confluence; 6. presence of melanocytes with nuclear atypia; 7. absence of maturation of melanocytes with descent into the dermis; 8. intradermal necrosis and degeneration of melanocytes; 9. lymphocytic infiltrate; 10. desmoplasia and ulceration. There were 22 male (56.4%) and 17 female (43.6%) patients.

- Koyama T, Ogawa M, Kurata S, Komazawa M, Murakami M.
Meningeal malignant melanoma in a child: immunocytological diagnosis. Acta Paediatr Jpn 34:173-178, 1992.
Abstract : A 10 year old boy, who was thought to have had a traumatic intracranial hemorrhage, was transferred to our Children's Medical Center. In spite of conservative treatment, he developed dysarthria, systemic convulsions, unconsciousness, quadriplegia, and consecutive paralysis of the cranial nerves. Magnetic resonance imaging scans demonstrated areas of increased signal intensity around the brain stem. The cerebrospinal fluid (CSF) contained a few large cells with abundant melanin-like granules, and numerous bizarre cells. The latter were considered to be malignant melanoma cells on immunocytological examination. Chemotherapy with dimethyltriazenoimidazole carboxamide (DTIC) and interferon beta (IFN-beta) was ineffective and he expired. Autopsy revealed diffuse infiltration of malignant melanoma cells into the meninges. We think that immunocytological examination of CSF is advisable for correct and rapid diagnosis.

- Larsson BS.
Melanin-affinic thioureas as selective melanoma seekers. Melanoma Res 1:85-90, 1991.
Abstract : 2-Thiouracil and some related thioureas are receiving growing interest as selective melanoma seekers. They are incorporated into growing melanin, apparently due to covalent binding to dopaquinone, and the adduct is gradually trapped in the melanin polymer during its formation. To be clinically useful in melanoma scanning, thiouracil has been radioiodinated, and 5-iodo-2-thiouracil (ITU) was found to be localized in melanotic melanoma as selectively as thiouracil. Clinical trials with ITU, for the detection of malignant melanoma, are in progress, and the results so far are promising. Treatment with [35S]thiouracil has been performed on melanoma-bearing mice. The radiodoses needed for cure, however, were very high, which makes clinical application hazardous. Boron neutron capture therapy, on the other hand, might be a better approach. The technique is based on the irradiation of tumours with slow neutrons from an external source after the accumulation of boron in tumour tissue and clearance from normal tissues. Boron-10 undergoes instantaneous nuclear fission through the reaction 10B(n,alpha)7Li, and the emitted particles are efficient in cell killing. Boronated thioureas have been synthesized in various laboratories, and data from experiments on melanoma-bearing mice indicate that some of these compounds accumulate in the tumours in concentrations necessary for successful treatment.

- Maloney ME, Jones DB, Sexton FM.
Pigmented basal cell carcinoma: investigation of 70 cases. J Am Acad Dermatol 27:74-78, 1992.
Abstract : Pigmented basal cell carcinoma (PBCC) is a clinical and histologic variant of BCC. Our purpose was to identify the histologic subtypes of BCC that were most often associated with pigment and to determine whether this correlated with outcome after excision. A series of PBCC was identified and the histologic subtype noted. Margins of all excisions were examined for residual tumor. These results were then compared with a series of nonpigmented BCCs. In a series of 1039 consecutive

BCCs, 70 (6.7%) contained pigment. The histologic growth pattern most frequently associated with pigment was the nodular/micronodular pattern (12.4%) followed by the nodular (7.7%), superficial (7.2%), micronodular (4.0%), and the nodular/micronodular/infiltrative (3.4%) patterns. Margins were examined for evidence of residual tumor in the 40 cases that were excised. In only one case (2.5%) was the margin positive for tumor. This was statistically significant ($p < 0.05$) compared with 388 excisions of nonpigmented BCCs with comparable growth patterns in which 69 (17.7%) showed positive margins. PBCC, as a clinical variant, is more frequently excised with adequate margins than are tumors of comparable histologic subtypes that do not contain pigment.

- Nelson JS, Applebaum J.
Treatment of superficial cutaneous pigmented lesions by melanin-specific selective photothermolysis using the Q-switched ruby laser. Ann Plast Surg 29:231-237, 1992.
Abstract : The Q-switched ruby laser at 694 nm, a wavelength well absorbed by melanin relative to other optically absorbing structures in skin, causes highly selective destruction of pigment-laden cells. In addition, the 20-nsec pulse duration produced by this laser approximates the thermal relaxation time for melanosomes, thereby confining the energy to the target. This new laser system produces clinically significant fading of superficial cutaneous pigmented lesions in patients, without complications such as hypertrophic scarring or changes in the normal skin pigmentation, often seen with conventional laser systems or other therapeutic methods. In ongoing clinical trials at our facility, excellent results have been obtained for lentigines, cafe-au-lait macules, nevus spilus, Becker's nevi, and ephelides (freckles), without skin scarring or textural or permanent pigment changes. The purpose of this report is to (1) describe the theoretical considerations that can be understood and used by a nonlaser-oriented practitioner involved in achieving selective removal of superficial cutaneous pigmented lesions, and (2) describe the practical application of the device to the clinical management of patients.

- Nogita T, Nagayama M, Kawashima M, Hidano A, Kasori J, Morishima T.
Spitz naevus of the toe. Br J Dermatol 126:520-522, 1992.
Abstract : A 68-year-old Japanese woman presented with a brownish macule, containing two papules, on her left fourth toe. Histological examination revealed an intradermal epithelioid cell tumour with irregularly shaped, bizarre giant cells. In the upper portion of the tumour, the epithelioid cells contained abundant melanin. A low amount of 5-S-cysteinyldopa and a diploid DNA distribution histographic pattern were helpful in differentiating the lesion from malignant melanoma. This location of a Spitz naevus is exceptional.

- Ochiai H, Nakano S, Miyahara S, Goya T, Wakisaka S, Kinoshita K.
Magnetic resonance imaging of a malignant transformation of an intracranial cellular blue nevus. A case report. Surg Neurol 37:371-373, 1992.
Abstract : As a follow-up to a case previously reported, a rare case of malignant transformation of cellular blue nevus (CBN) in the central nervous system preoperatively diagnosed by magnetic resonance imaging (MRI) is reported. On MRI, the malignant portion of the nevus was slightly hyperintense on both T1- and T2-weighted images. In contrast, the benign portion with a great deal of melanin was hyperintense on T1-weighted image and hypointense on T2-weighted image. MRI was useful and indispensable for detecting the malignant transformation of CBN.

- Offner FA, Wirtz HC, Schiefer J, Bigalke I, Klosterhalfen B, Bittinger F, Mittermayer C, Kirkpatrick CJ.
Interaction of human malignant melanoma (ST-ML-12) tumor spheroids with endothelial cell monolayers. Damage to endothelium by oxygen-derived free radicals. Am J Pathol 141:601-610, 1992.
Abstract : Clinical and experimental observations suggest that tumor-induced endothelial cell injury may be one of several initial events in the establishment of tumor metastases. To test this hypothesis, the authors have analyzed the interaction of malignant melanoma (ST-ML-12) multicenter tumor spheroids with endothelial cell monolayers in a three-dimensional coculture system. After 1.5 hours of interaction, the authors observed a toxic effect on endothelial cells in the perispheroid region. The latter was demonstrated by testing membrane integrity with the fluorescent probes acridine orange/ethidium bromide and resulted in sensitivity to shear stress of the damaged cells. The

endothelium then underwent a regenerative cycle to replace the denuded halo. Addition of the oxygen radical-scavenging enzyme superoxide dismutase to the culture medium prevented this endothelial cell damage in a dose-dependent manner for up to 12 hours. By contrast, catalase, deferoxamine mesylate, allopurinol, and the proteinase inhibitors soybean trypsin inhibitor and aprotinin were not protective under the same conditions. The endothelial damage was dependent on the attachment of the spheroids. Medium conditioned by ST-ML-12-spheroids proved to be ineffective. A similar, but less prominent, deleterious effect was seen when human peritoneal mesothelial cells were used in place of the human umbilical vein endothelial cells. Spheroids of the uroepithelial cell line HU-609 were used as control. No toxicity was observed in these cocultures. Melanin biosynthesis is associated with the production of oxygen-derived free radicals. The results suggest a possible implication of these free radicals in metastasis formation of malignant melanoma.

- Pandit AA, Prayag AS.
Cytodiagnosis of metastatic melanoma in the lymph nodes. Indian J Cancer 28:185-187, 1991.
Abstract : Lymph-node aspirates performed over ten years numbering 1,555 showed 8.2% of metastatic tumors. Of these, only six percent were metastatic melanomas. Clinical diagnosis was made in three cases. Melanin in the cells made the diagnosis easy. But even in the absence of the pigment as in two amelanotic melanomas, the cytologic features were characteristic.

- Ristic A, Djordjevic-Markovic R, Pavlovic M, Krsmanovic V, Kanazir DT.
The effect of glucocorticoid and antiglucocorticoid hormones on the growth of mouse melanoma cells. Anticarcinog Radiat Prot 2, Proc Int Conf, 3rd Meeting 1989, 401-403. (Nygaard OF, Upton AC, eds) Plenum, New York, NY, 1991.
Abstract : Melanoma cell growth was stimulated by the glucocorticoid triamcinolone acetonide; growth was inhibited by the antiglucocorticoid cortexolone or progesterone and by the coordination of triamcinolone and cortexolone. Melanin secretion was stimulated by triamcinolone and also by progesterone; cortexolone inhibited melanin secretion, both alone and in combination with triamcinolone. The differing biological response to cortexolone and progesterone suggest that they act through sep. mol. mechanisms. However, triamcinolone and cortexolone may act via the same receptors.

- Rustin GJ, Stratford MR, Lamont A, Bleehen N, Philip PA, Howells N, Watfa RR, Slack JA.
Phase I study of intravenous 4-hydroxyanisole. Eur J Cancer 28A:1362-1364, 1992.
Abstract : 4-Hydroxyanisole is a depigmenting agent which has been shown to have activity against malignant melanoma when given intra-arterially in man. An intravenous dose escalation study has been carried out with the aim of obtaining maximum plasma concentrations in a 5 day schedule. 8 patients entered this study which was stopped because of drug toxicity after 3 patients had been treated at the third dose escalation of 15 g/m2. 2 patients had WHO grade 4 liver and one also grade 4 renal toxicity and another had grade 4 haemoglobin toxicity. Extrapolated plateau plasma levels between 112 and 860 mumol/l were obtained, which in vitro studies suggested would be cytotoxic. Hopefully, newer analogues will have a greater specificity for the melanin pathway with less toxicity.

- Seki Y, Ohara K, Aiba T, Unakami M, Hara M.
Primary intracranial amelanotic melanoma--report of an autopsy case. Neurol Med Chir (Tokyo) 31:773-776, 1991.
Abstract : Primary intracranial amelanotic melanoma was verified at autopsy in a 38-year-old male. Correct diagnosis of amelanotic melanoma needs electron microscopy or immunohistochemistry, since Masson staining is negative due to the absence of melanin pigment. We adopted the following criteria for clinical use: macroscopically not dark and microscopically negative for Masson staining, but ultrastructurally various melanoma types present. Although the clinical profile of this case is consistent with melanotic melanoma, the more detailed features of primary intracranial amelanotic melanoma require future study.

- Supino R, Mariani M, Colombo A, Prosperi E, Croce AC, Bottiroli G.
Comparative studies on the effects of doxorubicin and differentiation inducing agents on B16

melanoma cells. Eur J Cancer 28A:778-783, 1992.
Abstract : The differentiation-inducing activity of doxorubicin on B16 melanoma cells grown in vitro was compared with that of other known differentiation inducers, such as theophylline, retinoic acid, and melanocyte-stimulating hormone (MSH). At drug concentrations resulting in cytostatic effects, doxorubicin and theophylline induced morphological changes (dendritic-like structures with a terminal melanin granule) with an enhancement of total melanin content and tyrosinase activity. Retinoic acid did not alter melanin content and cell morphology, although it affected cell growth. MSH enhanced total melanin content and tyrosinase activity, with no significant morphological changes. Flow cytometric analysis showed that MSH led to an accumulation of cells in G1 phase whereas doxorubicin induced an accumulation of cells in G2 + M. Studies on DNA content in doxorubicin-treated cells, selected on the basis of a morphologically differentiated pattern, showed a clustering of these cells in G2 + M, probably due to a cytokinesis block. Thus doxorubicin can induce cell differentiation comparable with other differentiation inducers.

- Taylor CW, Grogan TM, Lopez MH, Leong SP, Odeleye A, Feo-Zuppardi FJ, Hersh EM.
Growth and dissemination of human malignant melanoma cells in mice with severe combined immune deficiency. Lab Invest 67:130-137, 1992.
Abstract : The severe combined immune deficiency (SCID) mouse is lacking mature B and T lymphocytes and may be permissive for human tumor growth and metastasis. SCID mice received human melanoma cells of diverse origins including: 2 established cell lines, 4 early passage cell lines, and fresh or cryopreserved cells obtained directly from 9 patient biopsies. They were introduced into SCID mice via intraperitoneal, subcutaneous and intravenous injections. Tumor growth occurred with each of the 15 melanoma specimens for a take rate of 100% considering cell source. In addition, 60% of the 102 total mice injected displayed tumor growth in at least one site. The most consistent tumor growth (77%) occurred after intraperitoneal injection. Tumors developed in 41 and 48% of mice injected subcutaneously and intravenously, respectively. The mice developed both local tumor growth with palpable tumor nodules at injection sites and hematogenous and/or lymphatic dissemination to multiple sites in the abdominal and thoracic cavities. The number of metastases per animal averaged 16.3 and the number per organ ranged from 1 to 38. Melanotic and amelanotic tumor nodules obtained from a single patient retained their original characteristics with regard to melanin production after passage in the SCID mouse. The appearance of the human melanoma cells in SCID mouse tissues ranged from implants on the organ capsule to frank parenchymal organ involvement and vascular invasion. Some small foci of tumor were only detected using immunohistochemistry with monoclonal antibodies against the S-100 and HMB-45 to melanoma-related antigens. We conclude that the SCID mouse consistently supports growth, invasion, and metastatic spread of human melanoma cells, including specimens obtained from fresh patient biopsies. The SCID mouse will serve as a relevant in vivo model for studying the biology of human malignant melanoma and screening new therapeutic agents.

- Tsukamoto K, Gersten DM, Law LW, Hearing VJ.
Malignant melanoma: relationship to parameters of differentiation. Melanoma Res 1:223-230, 1991.
Abstract : Malignant melanoma is not only one of the most aggressive and lethal types of neoplasm, but its incidence in the general population is currently increasing at an alarming pace. It is interesting that most melanomas retain many of their characteristics of differentiation, including a dendritic nature and the production of melanin. This review discusses the phenotypic properties of melanoma cells, including their state of differentiation, and their tumourigenic and metastatic potentials, and attempts to provide an overview of the state of current research on the interrelationships between those parameters in murine and human systems.

- Wakamatsu K, Ito S, Horikoshi T.
Normal values of urinary excretion and serum concentration of 5-S-cysteinyldopa and 6-hydroxy-5-methoxyindole-2-carboxylic acid, biochemical markers of melanoma progression. Melanoma Res 1:141-147, 1991.
Abstract : The urinary excretion of the pheomelanin precursor 5-S-cysteinyldopa (5-S-CD) has been used as a tumour marker for metastatic melanoma. The eumelanin-related metabolite 6-hydroxy-5-

methoxyindole-2-carboxylic acid (6H5M12C) is also excreted at high levels in some melanoma patients. In order to compare normal values, we measured urinary excretion and serum concentration of 5-S-CD and 6H5M12C in 33 Japanese normal subjects. The mean values of 5-S-CD and 6H5M12C in urine were 0.45 and 0.39 mumol/day, respectively, and those in serum 4.3 and 3.6 nmol/l. Levels of these markers in urine were much more variable than those in serum. We have adopted 1.5 mumol/day and 10 nmol/l as the upper limits for normal ranges of the urinary excretion and serum concentration, for both markers. No significant differences were found between men and women. There were no correlations among the four markers. The urinary excretion of both markers showed significant decrease in elderly subjects as compared with middle-aged subjects, while the serum concentration showed no age-dependent differences. These results suggest that the levels of 5-S-CD and 6H5M12C in serum are more reliable as tumour markers for the estimation of melanoma progression than those in urine.

- Yin M, Yoshida MA, Tonomura A, Kasuga T.
Cytogenetic studies of human malignant melanoma cell lines. Bull Tokyo Med Dent Univ 39:43-54, 1992.
Abstract : Cytogenetic studies were performed on six cell lines derived from three patients suffering from malignant melanomas. The modal chromosome numbers were in the hypotriploid to hypertetraploid ranges and both the numerical and structural aberrations of chromosomes were found. Aberrations were most frequently observed in chromosomes 1, 6 and 7. Deletion of 1q was consistently present in all cell lines, while loss of 6q was observed in two cell lines of case 1. Translocations t (Y; 6) and t (6;?) occurred in one cell line from case 3. An increased number of copies of chromosome 7 was a characteristic feature of the cell lines from case 2. Since positive correlation between the expression of EGF receptors and an increased dosage of chromosome 7 has been reported for malignant melanomas and the gene for EGFR has been mapped to band 7p12-p13, this phenomenon might be of importance for the proliferation of malignant melanoma. The findings of the present study are generally in agreement with the data previously published in the literature, indicating the existence of specific non-random chromosome lesions during melanoma development.

9. Eye

- Engbretson GA, Linser PJ.
Glial cells of the parietal eye: structural and biochemical similarities to retinal Muller cells. J Comp Neurol 314:799-806, 1991.
Abstract : The parietal eye of lizards is a relatively simple yet highly structured visual organ. Cone-like photoreceptors synapse directly onto ganglion cells that project to the brain. Golgi staining confirmed the existence of glial cells spanning the sensory epithelium in a manner analogous to the Muller cells of the vertebrate lateral eye retina. These parietal eye glial cells bear expanded end feet at the basal border of the eye. Electron microscopic examination revealed some ultrastructural similarity to Muller cells. Mitochondria, osmiophilic and transparent vesicles, and the Golgi apparatus are found in the apical end of the parietal eye glial cell. Junctional complexes join adjacent parietal eye glial cells and their neighboring photoreceptor cells. Bundles of filaments are found in the basal end of the cell and the plasma membranes of adjacent cells often intertwine tightly in this region. The parietal eye glial cells are immunoreactive to antibodies against glutamine synthetase, also characteristic of Muller cells. Some differences between parietal eye glial cells and Muller cells also are evident. The parietal eye glial cells are not immunoreactive to antibodies against another Muller cell marker, carbonic anhydrase II, and many of them contain melanin granules, while Muller cells are not pigmented. In addition, we have found that the lens cells of the parietal eye and the Muller cells of the lateral eye retina of lizards are immunoreactive to antibodies against both glutamine synthetase and carbonic anhydrase II.

- Fukuda M, Sasaki K, Jin U.
The uptake of fluoroquinolones in the iris-ciliary body. Atarashii Ganka 9:1204-1207, 1992.

Abstract : After 0.3% sparfloxacin (SPFX) solution was topically administered to pigmented and albino rabbit eyes continuously for 7 days, the SPFX concentration in the iris-ciliary body was monitored for 4 wk. SPFX couldn't be detected in the albino rabbit iris-ciliary body at 24 h; however, 1.71 ± 0.40 μg/gwas retained in the iris-ciliary body of the pigmented rabbit eye for 2 wk. The results indicate that there is a relationship between the intraocular dynamics of fluoroquinolones and melanin. The uptake and release of ocular antibiotics must be studied with regard to both their short- and long-term dynamics.

- Hu DN, Ritch R, McCormick SA, Pelton-Henrion K.
Isolation and cultivation of human iris pigment epithelium. Invest Ophthalmol Vis Sci 33:2443-2453, 1992.
Abstract : There have been very few attempts to isolate and culture human iris pigment epithelium (IPE). Earlier efforts that used whole iris explant methods did not achieve pure cultures of IPE. We have developed methods for separating the IPE from the iris stroma of post-mortem eyes that avoid contamination by other cell types. Three different isolation methods were studied: direct dissection, enzyme digestion, and enzyme-assisted microdissection. The latter method yielded the best results. After treatment with enzyme solution, the IPE was easily separated from the stroma under the stereomicroscope and subsequently cultured with supplemented F12 medium. With this method, approximately 2.3 x 10(5) cells were isolated from each iris with an average viability of 90.2%. IPE cells isolated from 19 of 24 eyes grew to confluence in primary culture. The IPE could be maintained in pure culture for many generations over several months with up to 20 population doublings. Cultured IPE demonstrated cytokeratin and S-100 protein by immunocytochemistry. Some of these cells also displayed desmin, indicating origin from the anterior IPE. Cultured IPE cells retained most of the characteristics of IPE in vivo, such as apical/basal polarization, microvilli, and many cell junctions. Gradual dilution of pigment occurred in the dividing IPE cells, suggesting an inability to produce melanin in vitro. A subpopulation of the IPE cells contained myofilaments by electron microscopy, also indicating a anterior IPE origin. This method provides a source for large numbers of human IPE cells and could be useful in studies of the biology of IPE and the role of IPE in pathogenesis of several eye diseases, most notably exfoliation syndrome and its associated glaucomas.

- Kaiser PK, Pineda R, Albert DM, Shore JW.
'Black cornea' after long-term epinephrine use [clinical conference]. Arch Ophthalmol 110:1273-1275. 1992.
Abstract : Fifteen years after a partial maxillectomy and radiation therapy for left antral carcinoma, a 53-year-old woman presented to the Eye Plastics and Orbit Service of the Massachusetts Eye and Ear Infirmary, Boston, with phthisis and a large, black corneal lesion in the left eye. She had been treated for unilateral glaucoma in the left eye for more than 10 years with topically administered epinephrine borate, timolol maleate, and pilocarpine hydrochloride. Clinically, the lesion was smooth, black, and homogeneous, and was thought to represent uveal prolapse covered by a thin layer of epithelium. An eyelid-sparing anterior exenteration was performed. Histopathologic examination revealed an acellular, homogeneous substance that stained positively with the Fontana Masson stain for melanin and bleached with potassium permanganate, findings consistent with corneal adrenochrome deposition. Since adrenochrome can be easily dissected free from the cornea, this case illustrates that misdiagnosing adrenochrome deposition may lead to unnecessary surgery.

- Menon IA, Wakeham DC, Persad SD, Avaria M, Trope GE, Basu PK.
Quantitative determination of the melanin contents in ocular tissues from human blue and brown eyes. J Ocul Pharmacol 8:35-42, 1992.
Abstract : The amount of melanin in the iris, ciliary body, and retinal pigment epithelium-choroid of human subjects was separately determined. The results are expressed as the amount of melanin/mg tissue as well as the amount of melanin in the whole tissue. The results showed that there was no statistically significant difference between the melanin content of the iris in blue and brown eyes. However the ciliary body and retinal pigment epithelium-choroid from brown eyes had more melanin than the corresponding tissues from blue eyes. Blue and brown eyes with higher color intensity had more melanin than the corresponding eyes with lesser intensity of color. The differences

between brown and blue eyes in their melanin content may have relevance to the pharmacokinetics of drugs that bind to melanin. This would mean that the larger amounts of melanin would decrease the initial levels of the drugs and would increase the drug levels after prolonged periods.

- Tetsumoto K, Schlotzer-Schrehardt U, Kuchle M, Dorfler S, Naumann GO.
Precapsular layer of the anterior lens capsule in early pseudoexfoliation syndrome. Graefes Arch Clin Exp Ophthalmol 230:252-257, 1992.
Abstract : We examined a possible correlation between clinical signs of early pseudoexfoliation (PSX) syndrome related to pigment dispersion and iris stroma atrophy and morphological alterations of the lens capsule. 63 anterior lens capsules (30 PSX suspects, 3 pre-PSX, 10 PSX, 20 controls) were studied by transmission and immuno-electron microscopy (TEM). In 20 PSX suspect and 3 pre-PSX capsulotomy specimens, TEM revealed a precapsular layer (0.1-11 microns in thickness) composed of microfibrils, amorphous material, and granular inclusions. The incidence of this fibrillar layer was significantly higher (p = 0.001) in PSX suspect and pre-PSX eyes than in controls (5 positive). Ultrastructural and immunohistochemical similarities of the fibrillar surface network in PSX suspect and typical PSX specimens indicate that the precapsular layer may represent a precursor of PSX. The beginning PSX process in the eye is obviously indicated by certain clinical signs.

- Yamaguchi K, Yamaguchi K, Young RW, Gaur VP, Greven CM, Slusher MM, Turner JE.
Vitreoretinal surgical technique for transplanting retinal pigment epithelium in rabbit retina. Jpn J Ophthalmol 36:142-150, 1992.
Abstract : Transplantation of retinal pigment epithelial (RPE) cells has been proposed as a potential remedial procedure for previously untreatable retinal diseases. In this study, a vitreoretinal surgical technique was used to transplant pigmented RPE cells obtained from pigmented rabbits into the subretinal space of New Zealand White rabbits. At the time the animals were sacrificed, the retina was re-attached in all but 4 of the 24 experimental eyes. Histologically, by one week the transplanted RPE cells had formed a monolayer in patchy areas beneath the attached retina. By electron microscopy, RPE cells with prominent melanin granules were found attached to Bruch's membrane. Three weeks after transplantation, grafted RPE cells had formed apical microvilli and tight junctions with adjacent cells. The nucleus of the cells containing pigment had become oval, and their contact with Bruch's membrane appeared to be composed of bsal infoldings that were well formed. Our findings demonstrated the functional appearance of the transplanted RPE cells.

10. Other

- Hepel M.
Characterization of conductive composite polymers by the EQCM technique. Ceram Trans 19:389-396, 1991.
Abstract : Novel composite polymer films based on conducting polpyrrole (I) matrix were synthesized. I films were doped with large bioorg. polymer mols., melanin (M). This type of a composite polymer film could function as a controlled release membrane. The ion dynamics in I-M films were studied in situ using linear scan voltammetry and microelectrogravimetry with piezoelec. quartz crystal microbalance (EQCM). The EQCM technique provided wealth of information when testing the irreversibility of incorporation of various dopants. The preferential cationic charge counterbalancing was observed in Na2SO4 solutions in the reversible potential range. Considerable changes in ion dynamics occurred upon the medium exchange. These changes were quite reversible and were due to the specific interactions of M with a variety of active ionic species in the solution.

- Phelan AM, Lange DG, Kues HA, Lutty GA.
Modification of membrane fluidity in melanin-containing cells by low-level microwave radiation. Bioelectromagnetics (NY) 13:131-146, 1992.
Abstract : The treatment of a B16 melanoma cell line with 2.45-GHz pulsed microwaves (10 mW/cm2, 10-μspulses at 100 pps, 1-h exposure; SAR, 0.2 W/kg) resulted in changes of membrane

ordering as measured by EPR reporter techniques. The changes reflected a shift from a more fluid-like phase to a more solid (ordered) state of the cell membrane. Exposure of artificially prepared liposomes that were reconstituted with melanin produced similar results. In contrast, neither B16 melanoma cells treated with 5-bromo-2-deoxyuridine (3 μg/day times 7 days) to render them amelanotic, nor liposomes prepared without melanin, exhibited the microwave-facilitated increase of ordering. Inhibition of the ordering was achieved by the use of superoxide dismutase, which strongly implicates oxygen radicals as a cause of the membrane changes. The data indicate that a significant, specific alteration of cell-membrane ordering followed microwave exposure. This alteration was unique to melanotic membranes and was due, at least in part, to the generation of oxygen radicals.

- Raaphorst GP, Azzam EI.
Response of transformed and normal mouse cell lines to anti-melanin compounds, hyperthermia, and radiation. Pigment Cell Res 5:25-29, 1992.
Abstract : Five cell lines (one parental, two transformed melanin producing, and two transformed non-melanin producing) were evaluated for the responses to 2- and 4-hydroxyanisole (2HA, 4HA) alone or combined with hyperthermia or radiation. All cells exhibited a non-specific toxic response to the two compounds and the effect was exposure time and concentration dependent and was greater for 4HA compared to 2HA. In addition, the two melanin-producing cell lines were more sensitive, demonstrating specific toxicity to such cell lines. The treatment with either 2HA or 4HA combined with heat and radiation resulted mostly in additive or antagonistic effects, except for one combination of 2HA plus radiation in the melanin-producing R25 cells. Thus, while these compounds may be useful in therapy for pigmented melanomas, combined treatment with radiation is not recommended.

- van der Zypen E, Fankhauser F, Luscher EF, Kwansniewska S, England C.
Induction of vascular haemostasis by Nd:YAG laser light in melanin-rich and melanin-free tissue. Doc Ophthalmol 79:221-239, 1992.
Abstract : Haemostasis was effected in vessels of melanin-rich (MR: choroid) and melanin-free (MF: mesentery) rabbit tissue irradiated with a cw-Nd:YAG laser. The following parameters were employed: - pulse duration: 200 ms (MR) and 100 ms (MF); focal spot diameter: 200 microns (MR) and 80 microns (MF); pulse energies: 100-250 mJ (MR) and 0.5-1 J (MF); irradiances: 1.6-4.0 kW cm-2 (MR) and 1-2 x 10(2) kW cm-2 (MF). In melanin-rich tissue, laser energy is absorbed principally by melanin granules contained within the stromal melanocytes. The heat generated in these structures radiates into the surrounding tissue where it is dissipated. The damage thus incurred by the endothelium of blood vessels encompassed within this field triggers the haemostatic mechanism whereby blood flow is arrested. This effect is realized by the formation of an occluding plug of platelets, which is stabilized by the deposition of fibrin, particularly in capillaries, and to a lesser degree in larger vessels of the vascular lamina. In melanin-free tissue, haemoglobin serves as the primary site of energy absorption, which is thus shifted from the stroma to the vessel lumen. Irradiation of vessels in such tissue leads to thermocoagulation of plasma proteins and consequent stasis of blood flow.

ANNOUNCEMENTS & RELATED ACTIVITIES

Melanins and Melanogenesis

Giuseppe Prota

Universita Degli Studi di Napoli Federico II
Dipartimento di Chimica Organica e Biological, Italy

October 1992, 277 pp., isbn 0-12-565970-9
Audience: Cell biologists, biochemists, research physicians and dermatologists, libraries, and medical libraries.
MARKETING CODES : 2100 2500 5200

This book focuses on all aspects of melanin pigmentation, providing a concise, comprehensive picture of new knowledge gained at the frontiers of research. It draws heavily on the authors's 30-year activity in the field and his continuing work with specialists of widely diverse disciplines. The core of the book deals with the structure, physicochemical properties, and biosynthesis of the major classes of melanin pigments, including neuromelanins. Other discussions include the biology of the various types of pigment producing cells, the structure and mode of action of tyrosinase, and the chemistry of urinary melanogens and their biomedical applications as metabolic markers of melanocyte activity, especially for the follow-up of malignant melanoma. Finally, the book considers progress in the photobiology and photochemistry of melanins, with special emphasis on the controversial role of these pigments in skin photoprotection. This book is ideally suited as a basic guide for newcomers, and a handy source of specific information for practioners in academic, medical and industrial settings.

CONTENTS : An Introduction to Melanin Research. Melanin Producing Cells. Tyrosinase. Natural and Synthetic Melanins. Eumelanins. Neuromelanins. Pheomelanins and Trichochromes. Pigment Cell Metabolism. Enzymatic and Chemical Control. Genetic and Hormonal Regulation of Melanogenesis. Photobiology and Photochemistry of Melanogenesis. Chapter References. Subject Index. List of Abbreviations.

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Melanoma '93

Brighton Conference Centre, East Wing, 5-7 May 1993

The *Royal College of Pathologists* and the *Melanoma Study Group*, with the *South East Thames Regional Health Authority* and the *Brighton Health Authority* present a consensus conference to examine and discuss diagnostic issues associated with melanoma and other pigmented skin lesions. The conference will be of interest to any dermatologist, dermatopathologist or histopathologist, consultant or trainee, involved in the diagnosis or management of patients with melanoma.

Conference Programme

The Consensus Conference will address problems in the dermatopathology of melanoma. The conference will include formal presentations describing criteria from the published literature and pointing out areas of uncertainty. In line with previous Brighton conferences informality will be the key to a memorable meeting. A workshop atmosphere will prevail. A feature will be a large number of biopsy cases that will be available for inspection on microscopes. Some of the cases will have been submitted by delegates and will have been circulated to a panel of experts before the meeting. The biopsies will be presented and discussed in Slide Seminars. Although mainly aimed at dermatologists, dermatopathologists and histopathologists, the programme will be of interest to other specialists involved in the diagnosis or management of patients with pigmented skin lesions.

Sections of submitted cases and abstracts for free presentations on any subject related to the diagnosis or treatment of melanoma will also be accepted. The best abstracts will be given orally and others will be presented as posters. The deadline for submissions is 1st March 1993.

The conference will end with a series of discussions to form a consensus and to launch the MSG Trial of limited excision margins in melanoma.

A spouse programme will be available. Hotel accommodation and secure car parking will be available at discount rates. The conference takes place during the Brighton Festival. A dinner will be held on the Thursday evening of the meeting.

Speakers

Speakers will include:

- R L Barnhill, Boston
- K Blessing, Aberdeen
- D W K Cotton, Sheffield
- M G Cook, Guildford
- D E Elder, Philadelphia
- N Kirkham, Brighton
- T Krausz, London
- W J Mooi, Amsterdam
- J A Newton, London
- N P Smith, London

For further information contact :

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Dept of Histopathology
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Brighton BN2 5BE, U.K.
Fax +44-273-600182

Wednesday 5th May - Prologue Course

The **Prologue** course, run by Dr Karen Blessing, will be held from 9.30 am at the Old Ship Hotel and will allow a limited number of registrants to be introduced to a range of melanocytic lesions. A box of sections will be circulated beforehand. These biopsies will orientate delegates for the debate to follow and will provide a useful record for future reference.

International Colloquium on Neuromelanin and Parkinson's disease

Sorrento, Naples May 6-8, 1993

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Outline of Colloquium Schedule

Friday, May 7, 1993

Key-note Addresses

*Session I : **Structure, Biosynthesis and Physicochemical Properties of Neuromelanin***

*Session II : **Mechanisms of Degeneration of Pigmented Neurons***

Poster Session

Saturday, May 8, 1993

*Session III : **Etiological Factors of Parkinson's Disease***

*Session IV : **Experimental Models and Pharmacology : Clinical Perspectives***

Poster session

MAIN TOPICS

Within the main themes of the scientific sessions, discussions will be expanded to cover a broad range of relevant topics. These include:

. Structure and biosynthesis of epidermal melanin pigments
. Oxidative metabolism of catecholamines
. Mechanism of action of MPTP and other neurotoxins
. Inhibition of MAO
. Metal ion distribution in dopaminergic regions of human brain
. Neuronal tissue cultures
. Genetic bases of Parkinson's disease

XV INTERNATIONAL PIGMENT CELL CONFERENCE

26-30 September, 1993 - London, U.K.

"Don't forget ! Mark it in your diary now !

The XVth IPCC will be held in London on September the 26th - 30th, 1993.

Further information :

Prof. P.A. Riley
Division of Molecular Pathology
University College and Middlesex School of Medicine
The Windeyer Building
Cleveland Street
London W1P 6DB
Tel +44-71-380.9323
Fax +44-71-387.3310

This is the first IPCC to be held under aegis of the International Federation of Pigment Cell Societies and under the auspices of the ESPCR. We hope that you are coming to the Meeting to ensure its success."

ESPCR Patrons

To establish closer cooperation links and to strengthen the partnership between industrial environment and basic and clinically oriented investigators in the field of pigmentation, the ESPCR invites those prospective companies who are concerned to become Patrons of the Society. Sponsorship of this kind will be a momentous contribution to cover the costs contingent for the expansion of the activities and initiatives of the ESPCR while maintaining the membership fee at a modest level. We have every confidence that both supporting industries and the Society will greatly benefit from this privileged cooperation, which will give new impetus to research on pigmentation.

We recognize with appreciation the following companies who have supported the efforts and continued success of the ESPCR :

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NEWS FROM THE ESPCR

The ESPCR is delighted to welcome the following colleagues to membership and hope that they will play a full and active part in the Society.

Dr. ROSEI M.A.
University of Rome "La Sapienza"
Dept of Sci Biochemistry
Piazzale Aldo Moro 5
I - 00185 Rome

Dr. RACHKOVA M.
Dept of Biochemistry
Lab of Mol Oncology
Medical Institute
Armeiska str II
Stara Zagora 6003
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SURVEY OF CURRENT PIGMENT CELL RESEARCH IN EUROPE
1991-1992

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ANNOUNCEMENTS

I wish to announce the following :

MEETING ☐

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