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Editor: Professor F. Serri, Foundation for research in Dermatology, Catholic University, Largo A. Gemelli 8, 00168 Rome, Italy. Phone: 06-338.54.51 Fax: 06-305.13.43
Editorial Office: Dr G. Ghanem (Assit. Edt), C. Henrotte (Production Assist.), Lab. of Oncology and Exp. Surgery, Institut J. Bordet, Rue Heger-Bordet 1, 1000 Brussels, Belgium. Phone: 32-2-539.23.43 Fax: 32-2-537.47.34

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Direttore Responsabile: Prof. Giuseppe Prota, Presidente ESPCR, Dip. di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, 80134 Napoli.
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Meeting report

C.R.C.-B.A.C.R.-A.I.C.R. International Workshop
Melanogenesis : its chemistry as a therapeutic strategy in melanoma

E.J. Land
C.R.C. Department of Biophysical Chemistry, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.

This Workshop brought together 60 scientists from 13 countries investigating mechanisms of melanogenesis and rational approaches towards using the melanogenic pathway as a means of targeting the cytotoxic therapy of malignant melanoma. The organisers were : Prof. D.G. Harnden and Dr E.J. Land (Paterson Institute for Cancer Research, Manchester), Prof. P.A. Riley (University College and Middlesex School of Medicine, London), Dr N. Thatcher (Christie Hospital, Manchester) and Prof T.G. Truscott (University of Keele).

The unique biochemical characteristic of melanocytes is the propensity to produce melanin. The meeting was convened specifically to examine recent advances in knowledge of melanogenesis and the possibility that there might be some way of exploiting the melanin-forming property of malignant melanocytes as a means treating melanoma. An improved understanding of the chemistry of melanogenesis might lead to the ability to manipulate such chemistry which could lead to a treatment of melanoma based on subverting the chemistry of melanogenesis.

The problem of melanoma was put in perspective by Prof. Rona MacKie (Glasgow, U.K.) who discussed the *epidemiology and pathogenesis of melanoma* and emphasized the importance of early detection, with the prognosis worsening rapidly for increasingly thick lesions due to disseminated disease. Epidemiological and case-controlled studies strongly implicate ultra-violet radiation, in particular its B component (280-315 nm), as a major aetiological factor in cutaneous melanoma. Those at greatest risk appear to be white-skinned subjects who have an indoor occupation but who indulge in intense sun exposure for short periods of time, e.g. on vacation. Additional risk factors include fair complexion and large numbers of benign melanocytic naevi. The unsatisfactory nature of *current therapy for disseminated melanoma* was reviewed by Dr

N. Thatcher (Manchester, U.K.) who gave an overview of the metastatic behaviour of melanoma and the relatively poor results of treatment with a range of cytotoxic agents, immunotherapy, and adjuvant treatments. Although in most clinical series there were a proportion of responses, the efficacy of current treatments for metastasizing melanoma is currently highly unsatisfactory and new strategies are urgently needed.

The classical Raper-Mason scheme for melanogenesis postulates a pathway involving, successively, tyrosine, dopa, dopaquinone, dopachrome, dihydroxyindoles, indolequinones ... leading to eumelanin, or phaeomelanin with the added involvement of cysteine. *Enzymes* are essential to some reactions in this process. Dr F. Solano (Murcia, Spain) described how the regulation of mammalian eumelanogenesis is mainly carried out not only by the well-known enzyme tyrosinase, but also by the more recently discovered enzyme dopachrome tautomerase. This enzyme is able to catalyze dopachrome tautomerisation into 5,6-dihydroxyindole-2-carboxylic acid (DHICA), thus preventing dopachrome decarboxylation taking place in the spontaneous rearrangement of dopachrome into 5,6-dihydroxyindole (DHI) at neutral pH. As o-diphenols, both DHI and DHICA are possible substrates of tyrosinase leading to the corresponding o-quinones. The relative concentrations of these dihydroxyindoles, and hence their o-quinones and the composition of the resulting eumelanin polymer, depends crucially upon the activity of both enzymes. This controlled polymerisation may be a natural mechanism to protect melanocytes against the known cytotoxicity of the decarboxylated indoles, which are more reactive than their 2-carboxylated counterparts.

The possibility that *peroxidase* could also be involved in later stages of the biosynthesis of eumelanins was discussed by Prof G. Prota (Naples, Italy). Although peroxidase cannot convert the monophenol tyrosine to the diphenol, dopa, and hence lead to melanin, evidence was presented showing that peroxidase is much more effective than tyrosinase in catalysing the oxidative conversion of DHI and DHICA to melanin pigments.

The *stimulation of tyrosinase* in melanoma cells by adrenoceptor agonists and by catecholic compounds was described by Prof H. Rorsman (Lund, Sweden), the aim being to try to develop ways of influencing the metabolism of catechols. An increase in tyrosinase activity could thus lead to the enhanced production of cytotoxic quinones. The stimulating and cytotoxic effects of isoprenaline, theophylline, terbutaline and DOPAC on IGR1 melanoma cells was discussed in terms of their abilities to penetrate the cells, modify cell proliferation and generate active oxygen species, including H_2O_2. Active oxygen species appear to increase tyrosinase activity in IGR1 cells. Moreover, both the activation of tyrosinase and the cytotoxic effects of catechols were found to be eliminated by catalase.

The melanocytes of normal humans and melanoma patients also contain the enzyme *catechol-O-methyl transferase (COMT)*. This enzyme methylates the indolic melanin precursors DHI and DHICA. Dr S. Pavel (Amsterdam, The Netherlands) described the detection of such indoles in media from human melanoma cell cultures. Most of these possess a methoxy group in positions 5 or 6 which prevents their oxidation to simple o-quinones. The methylation, which can be considered as a protective mechanism in melanocytes against intrinsically generated toxic o-hydroxy products, is

accomplished by intracellularly localised COMT.

Several contributions dealt with detailed aspects of the chemistry of early stages in melanogenesis and related processes. An *overview of quinone reactivity* with respect to melanin formation was provided by Dr J.M. Bruce (Manchester, U.K.). Although much of the chemistry of the melanogenesis pathway up to the dihydroxyindoles DHI and DHICA is fairly well understood, the subsequent stages, even those leading to species with comparatively low molecular weight, are not. The problem is compounded by the complexity of the oxidation of DHI (and DHICA), which can, in principle, lead not only to the corresponding ortho-quinone, but also to both a quinone-imine and a quinone-methide, all three of which may be in tautomeric equilibrium. Homo- and hetero-coupling reactions involving one or more of these tautomers, leading in the first place to a multiplicity of isomeric dimers, could be important in the polymerisation ultimately resulting in melanin.

Quinones and quinone-methides are also involved in the molecular mechanisms for *cuticular sclerotization* as described by Prof M. Sugumaran (Boston, USA). The exoskeleton of insects and other arthropods are hardened to protect their soft bodies by a process called sclerotization. During hardening, soluble structural proteins and chitin fibres are rendered insoluble by reaction with reactive species derived from enzymatic activation of catecholamines, such as N-acetyldopamine and N-ß-alanyldopamine. Based on the reactive species formed, two different mechanisms have been identified to acount for sclerotization reactions. They are quinone tanning and quinone-methide sclerotization. Although initially these two mechanisms were considered to be dependent of each other, the recent discovery of two new enzymes, quinone isomerase and quinone methide isomerase, led to a unification of these mechanisms.

The initial steps involved in sclerotization closely resemble the initial reactions observed during melanization. Both processes involve the initial enzymic oxidation of catechols to quinones, and quinone tautomerisation to quinone methides. The introduction of a double bond into the side-chain and the oxidation of the side-chain desaturated catecholamine parallels the aromatisation of dopachrome to dihydroxyindole, and the oxidation of dihydroxyindole to the corresponding quinone.

Details of the chemistry of the transition : *dopachrome --> dihydroxyindole* were described by Prof H. Wyler (Lausanne, Switzerland). Four protonated forms of dopachrome were identified, the 6-OH group deprotonating with a pK of 0.8, the carboxy group with a pK of 3.1 and the nitrogen with a pK of 9.1. At neutral pH, the only product of dopachrome decay is dihyroxyindole, whereas at high pH (> 10) it is exclusively the anion of dihydroxyindole-2-carboxylic acid.

The maturity of the technique of *pulse radiolysis* is such that one can now be confident enough to tackle problems as complex as the process of melanogenesis. Although *one-electron oxidation of DHI and DHICA* is not thought to be a part of the melanogenic pathway, the rapid disproportionation of such radicals is an excellent means of preparing high concentrations of the subsequent metastable intermediates, e.g. indolequinones, which are thought to be important components of the melanogenic pathway. Dr P. O'Neill (Chilton, U.K.) described the one-electron oxidation of a series

of hydroxy- and methoxy-indoles using pulse radiolysis. One-electron oxidation of dihydroxyindole in the pH range 5-10 yields the corresponding oxygen-centred indole semiquinone radical (pK 6.8). With hydroxylated mono-methoxyindoles, the corresponding methoxyindoloxyl radical is formed. Further methylation of the hydroxy substituents, as in dimethoxyindole, results in the stabilisation of the corresponding cation which, depending on the pH, deprotonates at N(1) to yield the nitrogen-centred indolyl radical, with a pK of 6.0.

With the exception of dihydroxyindole, the radicals were all found to decay bimolecularly to yield semi-permanent products which eventually decayed unimolecularly. In the case of dihydroxyindole, using very low pulse doses the radical was found to decay unimolecularly. The latter decay was assigned to a reaction of the radical with the parent dihydroxyindole, rate constant $\sim 10^6$ M^{-1} s^{-1}. This could be a component of the route for melanin polymerisation.

Prof T.G. Truscott (Keele, U.K.) described a closely similar study of the species resulting from pulse radiolytic one-electron oxidation of dihydroxyindoles and their methoxylated metabolites. Whereas there was good agreement with the results reported by the previous speaker on the initially formed one-electron oxidised radicals, differences were apparent in the nature of the reactions of some of the radicals and subsequent metastable intermediates. A possible *scheme for polymerisation to melanin* was proposed involving a reaction of a quinone-methide with water to produce a trihydroxyindole, which may itself then react successively with the methide to produce dimers, then trimers, etc.

Prof B. Kalyanaraman (Milwaukee, USA) described the application of the technique of *electron spin resonance spectroscopy* to the identification and characterisation of free radicals derived from melanin precursors. Complexed with diamagnetic ions, especially Mg^{2+} and Zn^{2+}, catechol(amine) semiquinones live 10^4 times as long as in the absence of metal, allowing their protonation-deprotonation reactions to be readily studied. The addition reactions of quinones with nucleophiles e.g. amino acids (proline and methionine), peptides and proteins were also studied via the radicals derived from such adducts.

It was the discovery some 20 years ago that tyrosinase is capable of oxidising substrates that are *structural analogues of tyrosine* which suggested the possibility of using the melanogenic pathway as a targeting strategy for melanoma chemotherapy. Prof P.A. Riley (London, U.K.) described a series of investigations on the mechanism of action of substituted phenols of which the lead compound is the well-known depigmenting agent 4-hydroxyanisole (4HA) which is oxidised to the corresponding orthoquinone. The mechanism of the cytotoxicity of the orthoquinone probably depends predominantly on the formation of adducts with important cellular thiol-containing proteins. The possibility that the action depends on the generation of active oxygen species by redox cycling has been excluded and there is little evidence that semiquinones are involved in the toxic process (see below). 4HA also exhibits direct toxic actions, including inhibition of ribonucleotide reductase, inhibition of mitochondrial electron transport, and other effects on cell physiology. Separation of these direct actions from the tyrosinase-dependent cytotoxicity would serve to amplify the therapeutic index against melanogenic cells, and

the results of tests on a series of derivatives of 4HA including a range of oxy-ethers and thio-ethers of differing chain length were presented. In discussion, Dr K. Schwabe (Berlin, Germany) reported that his group had synthesized over 100 analogues of 4HA and shown that the propyl oxy-ethers were therapeutically effective against tumour volume in melanoma-bearing animals, but had shown no effect on survival of the animals employed in these tests.

The results of studies on the *mechanism of cytotoxicity* and analogues were described by Dr E.J. Land (Manchester, U.K.). Using pulse radiolysis, semiquinones were generated from the corresponding hydroquinones chemically synthesized by Mr C.J. Cooksey. The semiquinones neither reacted with oxygen nor with trans-butanoic acid, a water-soluble model for unsaturated fatty acids. Consequently, it appears unlikely that redox cycling or the initiation of lipid peroxidation via semiquinones comprise the cytotoxic mechanism. The 3,4-quinones which form rapidly by disproportionation of the corresponding semiquinone were found to react rapidly with several thiols and with ascorbic acid. Nucleophilic addition of protein thiols to the quinone is thus a more probable mediator of cytotoxicity. 4-(n-propoxy)phenol, which possesses five times the *in vitro* tyrosinase-dependent cytotoxicity towards rat cells compared with 4HA, was found to behave almost identically as far as the reactivity of semiquinones and quinones was concerned.

A closely similar approach to targeted cytotoxicity was described by Prof K. Jimbow (Edmonton, Canada) who showed that 4-S-cysteaminylphenol and some analogues are selectively incorporated into murine melanoma tissue and into actively melanising hair follicles with *selective destruction of melanocytes* resulting in depigmentation of black hair follicles in mice, N-acetyl-4-S-cysteaminylphenol being the most potent inducer of selective destruction of follicular melanocytes. This compound may prove to be a useful anti-tumour agent judged by the data on subcutaneously inoculated B16 F10 melanoma cells in mice.

Dr P.G. Parsons (Queensland, Australia) reported that despite many promising *in vitro* demonstrations of antimelanoma activity by *redox active agents* such as catechols, and by drugs such as buthionine sulphoximine which depress cellular defences against oxygen radicals, there remain many problems regarding the successful application of this approach to melanomas *in vivo*. It may be possible to overcome the lack of potency and selectivity of such agents by using combinations of drugs. Such combination therapy, together with measures for minimising mutations rates, may also combat phenotypic instability leading to the development of drug resistance.

Dr B.S. Larsson (Uppsala, Sweden) described an approach towards the therapy of melanoma using *aminothiol compounds which are selectively incorporated* into newly-synthesized melanin by reacting with orthoquinones generated during melanogenesis. Various compounds of this class, including thiourea and 2-thiouracil, have been shown by autoradiography to be specifically taken up into melanising tissue including murine melanoma. Radioiodinated 5-iodo-2-uracil has been used in patients for melanoma screening and pilot studies on treatment with ^{35}S-thioracil have been carried out on melanoma-bearing mice. However, the radiation doses required for substantial effects using this radionuclide are extremely high, making therapeutic clinical application

hazardous. As an alternative, boron neutron capture therapy has been attempted, using boronated thioureas which are selectively incorporated into newly-synthesized melanin and the ^{10}B can then be activated to undergo nuclear fission by irradiatin of tumours with thermal neutrons from an external source.

Dr A.J. Winder (Oxford, U.K.) reported on the effects of L-tyrosine phosphate and cytochalasin D on induction of pigmentation in cells, with a view to the possibility of developing *differentiation therapy* for melanoma. Both agents produced a small increase in the amount of tyrosinase messenger RNA, although the amount observed was insufficient to account for the increase in enzyme activity.

The relationship between *abnormal melanosome structure* and cytotoxic phenomena was discussed by Dr J. Borovansky (Prague, Czechoslovakia). Electron microscope investigations have confirmed the presence of abnormal and incomplete melanosomes in human melanomas from epidermal and mucosal sites, in melanoma metastases and in the B16 mouse melanoma. For example, 90% of melanosomes in cutaneous melanomas had membrane defects. Evidence that this leads to significant leakage of reactive melanin precursors was furnished by raised free radical-mediated lipid peroxidation in the liver of B16 melanoma-bearing mice.

Prof T. Sarna (Krakow, Poland) discussed the *photoactivation of melanin* and the possible generation of cytotoxic products resulting from it. Direct photo-oxidation of melanin was accompanied by oxygen consumption and the production of hydrogen peroxide. This process was shown to be strongly wavelength- and pH-dependent and was also influenced by the presence of metal ions. Photo-oxidation of melanin in the presence of photosensitizers was also reported, and the possibility of covalent binding of photosensitizers to melanin as an approach to photodynamic therapy was discussed. Dr A.R. Young (London, U.K.) discussed the apparently paradoxical *photoprotection* of skin to UV-induced damage by prior exposure to photosensitizers such as psoralens. Tans induced by 5-methoxypsoralen (5-MOP) contained in a UVB sun-screen with solar stimulating radiation (SSR) were found to reduce unscheduled DNA synthesis following minimal erythema doses of SSR. Despite the fact that 5-MOP is a weak photocarcinogen, judicious use of 5-MOP-containing sun-screens may offer benefits with regard to protection against various forms of light-induced skin cancer which could outweigh the risk involved. This may be of importance in protecting future generations from the risk of developing malignant melanoma.

The Workshop generated much detailed discussion which it is hoped will be reflected in the stimulation of new initiatives towards the introduction of a successful treatment for disseminated melanoma. Clearly one potentially powerful strategy is targeted cytotoxicity utilising the melanogenic pathway to activate an otherwise innocuous pro-drug.

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Pharmaceuticals, Farillon Ltd., Institut de Recherches Internationales Servier, Laboratoires Pharmaceutiques Bergaderm SA, Lilly Industries Ltd., l'Oréal, Sterling Drug Inc., US Air Force Office of Aerospace Research and Development, and Windsor Phamaceuticals Ltd.

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Review

Melanin affinity of toxic and carcinogenic substances

Bengt S. Larsson and Nils G. Lindquist
Dept of Toxicology, Biomedical Center, Uppsala University
Box 594, S-75124 Uppsala, Sweden

Using whole-body autoradiography and in vitro binding studies, we have investigated the melanin affinity of a large number of compounds (1-5). It is now well established that melanin has the property to accumulate several compounds such as various amines and probably also a number of metals, and to retain these agents, often for a very long time, even more than a year after a single administration (6). The physiological significance of melanin binding is however still poorly understood. One possibility is that melanin may protect the cells that harbour the pigment by keeping potentially harmful substances bound and slowly releasing the agents in low, nontoxic concentrations. The presence of melanin in some very sensitive tissues favours such a hypothesis. In the eye, and in the inner ear, melanin is located close to the receptor cells, in the retinal pigment epithelium and in the stria vascularis of the cochlea. These pigmented epithelial cells are involved in the nourishment of the receptor cells. In the brain, melanin is present in neurons in the extrapyramidal system, mainly in the substantia nigra and the locus coeruleus. However, this possible protection mechanism may under certain cicumstances be a threat to the cell. Thus may a long term exposure to a toxic compound with melanin affinity ultimately cause lesions in the cells. This mechanism seems to be an important factor in the development of some drug-induced ocular and inner ear lesions, and drug- and manganese-induced parkinsonism (1,4,7). Examples of compounds with melanin affinity causing such lesions are phenothiazine derivatives (ocular lesions, parkinsonism), chloroquine (ocular and inner ear lesions)(1,8) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; parkinsonism) (4,5,9). A conspicuous feature in the pathogenesis of these lesions is the degeneration of the melanin-containing cells.

Pigmented cells often seem to be subject to early ageing (1). The graying of hair, i.e. degeneration in the melanocytes of the hair bulbs, is perhaps the most obvious example. Senile degeneration of melanin structures also occurs in the eye, the inner ear, and the substantia nigra. Such degeneration may be of importance in the etiology of certain forms of senile lesions, such as some types of cataract formation and retinal degeneration secondary to degeneration in the pigment epithelium, hearing loss

secondary to strial atrophy, and idiopathic parkinsonism caused by destruction of the pigmented neurons in the substantia nigra and the locus coeruleus. These pre-senile conditions in the eye, the inner ear and the brain stem may be connected with accumulation and retention of noxious substances in the melanin of cells with a critical function in these organs. The melanin in these cells is very stable with a slow, if any, turnover. Therefore, it is possible that accumulation and retention of toxic agents may go on for years or even decades. Previously, the attention to melanin affinity was mainly focused on drugs. But some studies have also been dealing with the binding of metal ions to melanin, largely in vitro (10-13), but also in experimental animals (7,11,14). The knowledge in this regard, however, is so far incomplete, and since may metals are potent poisons, we have started a series of experiments to investigate the uptake of various metal ions in pigmented tissues after chronic exposure.

It has been found that melanin affinity of certain compounds is very high compared with others, and the substances showing the highest affinity are mainly organic amines and metal ions (2,13,15). Melanin is a polyanion, rich in negatively charged groups such as carboxyl groups and semiquinones (16-18). Substances with cationic properties (e.g. amines and metals) are bound to the melanin by ionic interaction (15,19). Both aliphatic (20) and aromatic amines are bound, especially the polycyclic amines. In the latter case, the ionic interaction is probably strengthened by other attractions, such as van der Waal's forces at the apposition of the aromatic rings of the compounds and the indole monomers of the melanin (15). The involvement of charge-transfer interaction has also been indicated for certain electron-donating substances, e.g. chlorpromazine (15,19), as well as hydrophobic interaction in some cases (21,22). The binding is normally complex - Scatchard analyses have shown that more than one binding class is involved for individual substances, including metals (15).

A prominent feature of the lesions caused by substances with melanin affinity is that, as mentioned above, the histologic changes are found in the melanin-containing cells, and in adjacent tissues, such as receptor cells. The effects are mainly chronic and related to high dose/long-term exposure. The entire manifestation of the lesions may occur after cessation of the offending substance, even years after discontinuance of the exposure (23). It is also possible that various substances, which are retained in the pigmented tissues, may cause additive adverse effects. The histopathologic changes in the melanin-containing cells are usually characterized by enlargement of the cells and a marked increase of the melanosome number. Later, degenerative changes occur with release of melanosomes and other cellular debris, which migrate into surrounding tissues. In the eye, e.g., pigment deposits can be seen in the retina, and atrophy of the photoreceptors (for review of the melanin-related histopathology, see ref. 1). The mechanism behind the development of the lesions is possibly a combination of selective retention (i.e. melanin affinity) and toxicity. The melanin serves as a chemical depot, from which the stored chemicals slowly become released with prolonged increase of the cytoplasmic concentrations. The degenerative course is ultimately determined by the intrinsic toxicity of the individual substance. It has also been proposed that, in some cases, the interaction per se between a substance and the melanin might change the chemical properties of the melanin with alteration, or even loss, of its physiological functions - there are some evidence for a protective role of the melanin in pigmented cells, e.g. as a sink for free radicals or excited, and potentially harmful, species (for review, see ref. 2).

The melanin affinity of certain substances may also be connected with melanoma induction. The main etiological factor behind malignant melanoma is apparently UV exposure, combined with, e.g., various phenotypic risk factors. But some epidemiologic studies and results from animal experiments have indicated that also chemical carcinogenesis may be involved in the etiology of the disease (e.g. ref. 24). In autoradiographic distribution studies on mice it has been found that some carcinogenic substances are retained in melanin-containing tissues due to melanin affinity, e.g. aflatoxin B_1 (22), tobacco-specific N-nitrosamines, benzidine, polycyclic hydrocarbons (dimethylbenz(a)antracene and benzo(a)pyrene), and some food pyrolysis products (25). In screening studies on isolated hair melanin we have recently identified more than 30 additional carcinogenic compounds with melanin affinity in vitro (Larsson & Roberto, unpublished results). The results so far obtained should exhort a methodical examination of the possible connection between melanin affinity and malignant melanoma, both epidemiologically and experimentally. A problem in this regard is that most studies on chemical carcinogenesis have routinely been performed in albino animals, which are refractory to melanin-related risks - therefore the use of pigmented animals is necessary.

References

1. Lindquist NG: Acta Radiol (Stockh), Suppl 325:1-92, 1973.
2. Larsson B: Acta Universitatis Upsaliensis, 43:1-52, 1979.
3. Lindquist NG: Uppsala J Med Sci, 91:283-288, 1986.
4. Lindquist NG, Larsson BS, Lyden-Sokolowski A: Pigm Cell Res, 1:133-136, 1987.
5. Lyden-Sokolowski A: Acta Universitatis Upsaliensis, 63:1-52, 1990.
6. Lindquist NG, Ullberg S: Acta Pharmacol Toxicol 31, Suppl. 2, 1-32, 1972.
7. Lyden A, Larsson BS, Lindquist NG: Acta Pharmacol Toxicol, 55:133-138, 1984.
8. Dencker L, Lindquist NG: Arch Otolaryngol, 101:185-188, 1975.
9. Lyden A, Bondesson U, Larsson BS, Lindquist NG: Acta Pharmacol Toxicol, 53:429-432, 1983.
10. White LP: Nature, Lond, 182:1427-1428, 1958.
11. Bruenger FW, Stover BJ, Atherton DR: Radiat Res, 32:1-12, 1967.
12. Potts AM, Au PC: Exp Eye Res, 22:487-491, 1976.
13. Larsson BS, Tjälve H: Acta Physiol Scand, 104:479-484, 1978.
14. Tjälve H, Nilsson M, Larsson BS: Acta Pharmacol Toxicol, 51:147-153, 1982.
15. Larsson BS, Tjälve H: Biochem Pharmacol, 28:1181-1187, 1979.
16. Nicolaus RA: Melanins, Ed. Lederer E; Hermann, Paris, 1968.
17. Felix CC, Hyde JS, Sarna T, Sealy RC: J Am Chem Soc, 100:3922-3926, 1978.
18. Ito S: Biochim Biophys Acta, 883:155-161, 1986.
19. Potts AM: Invest Ophtalmol Vis Sci, 3:405-416, 1964.
20. Tjälve H, Nilsson M, Larsson BS: Acta Physiol Scand, 112:209-214, 1981.
21. Stepien KB, Wilczok T: Biochem Pharmacol, 31:3359-3365, 1982.
22. Larsson P, Larsson BS, Tjälve H: Fd Chem Toxic, 26:579-586, 1988.
23. Burns RP: New Engl J Med, 275:693-696, 1966.
24. Rampen FHJ, Fleuren E: Med Hypotheses, 22:341-346, 1987.
25. Larsson BS, Tjälve H, Larsson P, Roberto A, Brandt I, Bergman K: Abstract, 2nd Meeting of the ESPCR, Uppsala, Sweden, p 64, 1989.

CURRENT LITERATURE

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

- Aime S, Fasano M, Croombridge C.
Solid-state carbon-13 NMR characterization of melanin free acids from biosynthetic and natural melanins. Gazz Chim Ital 120:663-664, 1990.
Abstract : 13C solid-state CPMAS NMR spectroscopy is used to evaluate the effect of H2O2 on natural and biosynthetic melanins. The 13C spectrum of a biosynthetic melanin prepd. in the presence of catalase is quite different from the natural melanin. However, the reaction of H2O2 on the biosynthetic melanin affords a water-sol. product, melanin free acid, whose 13C spectrum shows similarities with the natural pigment. In addn., these results may be relevant to understanding the role of melanin in removing H2O2 from living systems.

- Allegri G, Arban R, Costa C, Biasiolo M, Curcuruto O, Pozzan A, Traldi P.
Fast atom bombardment mass spectrometry in the study of dopamine melanogenesis intermediates. Pigment Cell Res 3: 181-186, 1990.
Abstract : Fast atom bombardment was applied to the study of the intermediates of the reaction dopamine-tyrosinase after treatment with diazomethane. The identification of trimethoxyindoline, dopamine-o-quinone and dimethoxyindole was easily achieved by this ionization method, together with accurate mass measurements and collision experiments. The structures of these compounds are in agreement with those already hypothesized in studies on melanogenesis.

- Alviano CS, Farbiarz SR, De Souza W, Angluster J, Travassos LR.
Characterization of Fonsecaea pedrosoi melanin. J Gen Microbiol 137:837-844, 1991.
Abstract : The constituents of the melanin complex from mycelial forms of F. pedrosoi were partially characterized. The pigment was mainly accumulated on large alkali-extractable, electron-dense cytoplasmic bodies (melanosomes) and, apparently, on the outer layer of the cell wall as external deposits within verrucose outgrowths. Using electron microscopy and Thiery's periodate/thiosemicarbazide/silver proteinate staining method, glycogen-like particles were also detected at the periphery of the cells. Melanin constituents comprised arom. and aliph./glycosidic structures with a predominance of the latter. IR spectra showed the presence of hydroxyl, carbonyl, and carboxyl groups. The aliph./glycosidic moiety consisted of fatty acids and polysaccharides with protein, in a ratio protein/polysaccharide 1:15. Rhamnose, mannose, galactose, and glucose (in the ratio 1:2:4:3.5) were the constituents of the polysaccharide. Lipid components included even-numbered, satd., and unsatd. fatty acids (in the ratio 2:1) ranging from C16 to C18. Palmitic and oleic acids were the prominent fatty acids. Aspartic and glutamic acids, leucine, glycine, and alanine were the major amino acids. Nonpigmented cells of F. pedrosoi were studied for comparison with the pigmented forms: they did not accumulate acid-insol. precursors of melanin.

- Costantini C, Crescenzi O, Prota G, Palumbo A.
New intermediates of pheomelanogenesis in vitro beyond the 1,4-benzothiazine stage. Tetrahedron 46:6831-6838, 1990.

Abstract : Enzymic oxidn. of 5-cystein-S-yldopa (I) leads to a complex mixt. of oligomeric products, 2 of which were isolated by preparative HPLC and identified as diastereomers corresponding to the gross structure (II). Under acidic conditions, both compds. are rapidly converted into trichochrome F (III), a .DELTA.2,2'-bi-(2H-1,4-benzothiazine) pigment previously isolated by extn. of red hair and feathers.

- d'Ischia M, Napolitano A, Prota G.
Peroxidase as an alternative to tyrosinase in the oxidative polymerization of 5,6-dihydroxyindoles to melanin(s). Biochim Biophys Acta 1073:423-430, 1991.
Abstract : The ability of the peroxidase/H2O2 system to promote the oxidative polymerization of 5,6-dihydroxyindole (DI) and 5,6-dihydroxyindole-2-carboxylic acid (DICA) to melanin pigments was investigated in comparison with tyrosinase. commonly regarded as the sole enzyme involved in melanogenesis. In 0.025 M phosphate buffer at pH 6.8, tyrosinase (2.7 x 10(-3) U/ml) induced a smooth oxidation of 3.0 x 10(-5) M DI (initial rate = 4.4 x 10(-5) M/s) to give a complex mixture of products with the 2,4'-dimer I as the main component, whereas, under the same conditions, peroxidase (0.44 U/ml) and 1.2 x 10(-4) M H2O2 caused the instantaneous conversion of the substrate to a well-defined pattern of products, comprising the 2,4'-and 2,7'-DI dimers I and II, and the related trimers III and IV. When 3.0 x 10(-5) M DICA was used as the substrate, the difference in the effectiveness of the enzymes was much more pronounced. Thus, while peroxidase accomplished the fast oxidation of the indole, yielding the dimer V and the trimer VI as the main products, tyrosinase proved unable to induce more than a poor and sluggish reaction with an initial rate of 5.6.10(-6) M/s. These results raise the possibility that peroxidase, rather than, or in addition to, tyrosinase, may play a critical role in the later stages of the biosynthesis of melanins.

- Ichinose K, Sugimori M, Itai A, Ebizuka Y, Sankawa U.
Deoxygenation in the biosynthesis of aromatic polyketides. Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 32:17-24, 1990.
Abstract : The deoxygenation reaction involved in the biosynthesis of fungal melanin was investigated by using synthesis, theor. chem., and enzymol. Biomimetic synthesis of scytalone (I), a simple deriv. of tetralone, was reinvestigated. I was formed from 1,3,6,8-tetrahydroxynaphthalene (II) by NaBH4 in the presence of MeONa. The reactive species of this reaction was clarified as an asym. keto tautomer (III) by NMR spectroscopy. The structure of the most stable ionic species of II in MeONa-MeOH was studied by semiempirical and ab initio (Gaussian) MO calcns. It was suggested that the most stable species was an asym. keto-tautomeric trianion, which is in good agreement with the observations by NMR spectroscopy. Transition state structures and stabilities were simulated for the H- attack of the carbonyl carbons at C-1 and C-3 of III. The results suggested that C-3 was more favorable for the redn., which agreed with expt. The enzymic activities which reduce II and 1,3,8-trihydroxynaphthalene to I and vermelone, resp., were detected in a cell-free ext. from shake cultures of Phialophora lagerbergii. Both reductive activities were obsd. in the same enzyme fractions obtained by partial purifn. using (NH4)2SO4 pptn. and DEAE cellulose column chromatog.

- Jacobsohn MK, Byler DM, Jacobsohn GM.
Isolation of estradiol-2,3-quinone and its intermediary role in melanin formation. Biochim Biophys Acta 1073:1-10, 1991.
Abstract : We have reported previously that 2-hydroxyestradiol can be oxidized in the presence of catechol by mushroom tyrosinase, with a stoichiometric requirement of molecular oxygen (Jacobsohn, G.M. and Jacobsohn, M.K., 1984) Arch. Biochem. Biophys. 232, 189-196). It is then incorporated into melanin (Jacobsohn et al. (1988) J. Steroid Biochem. 31, 377-385). We now report on the isolation and characterization of the o-quinone as a product of the enzyme reaction from 2-hydroxyestradiol. The o-quinone was isolated from incubates and identified by its FTIR spectrum, in particular, by the appearance of a new band at 1652 cm-1, its migration in HPLC systems, its ultraviolet spectrum, its derivatization with phenylenediamine and comparison of these properties with the periodate oxidation product of the same substrate. The enzyme oxidation of the catechol estrogen was performed at 37 degrees C and did not require an activator; dopa at concentrations higher than 5 microM was inhibitory. At concentrations lower than 5 microM, dopa acted catalytically and was not consumed during the course of reaction. Ascorbic acid inhibited the reaction. The quinone exhibited both reversible and irreversible binding to performed melanin and to melanin actively synthesized by the enzyme. Incubation of 18 microM newly synthesized

[4-14C]estradiol-2,3-quinone with mushroom tyrosinase for 45 min at 37 degrees C in presence of 400 microM dopa showed incorporation (irreversible binding) of 6.3 +/- 0.3% of label into melanin produced during the course of reaction. Similar incubations for 45 min of pre-formed melanin prepared from 400 microM dopa showed incorporation of 4.4 +/- 0.2% of the label. Reversible binding was 10-times greater than incorporation for both actively synthesized and preformed melanins. In the absence of dopa or catechol, enzyme incubations of either 2-hydroxy-estradiol or its quinone did not yield melanin. Data suggest that estradiol-2,3-quinone is an intermediate in the incorporation of the catechol estrogen into melanin by tyrosinase.

- Kozik A, Korytowski W, Sarna T, Bloom AS.
Interactions of flavins with melanin. Studies on equilibrium binding of riboflavin to dopa-melanin and some spectroscopic characteristics of flavin-melanin complex. Biophys Chem 38:39-48, 1990.
Abstract : Natural melanins are photoprotective pigments that in mammals are principally found in the skin, hair, and eyes. Although the molecular mechanism of photoprotection of pigmented cells has not yet been established, several hypotheses have been proposed with melanin acting as a light filter, free radical scavenger, and quencher of electronically excited states of reactive intermediates. It can be expected that the detoxicating efficiency of melanin should be enhanced if the melanin and potentially cytotoxic species are brought close together. Such a situation may occur for a number of photosensitizing dyes that have the ability to bind to melanin. The interaction of melanin with flavins has been studied under strictly controlled experimental conditions. The equilibrium dialysis method has been employed to determine dissociation constants and the number of binding sites in melanin at pH 5-9. The data reveal that synthetic DOPA-melanin has two different classes of binding sites with dissociation constants of 10(-6) and 10(-5) M, respectively. The overall binding capacity of melanin, at pH 7, is 250 nmol RF/mg melanin. The amount of bound-to-melanin RF increases with pH. The absorption spectra of melanin complexes with RF and lumiflavin indicate that hydrophobic interaction may be involved in the binding of these flavins by melanin. No changes in flavin fluorescence have been detected after binding of flavin to melanin. It appears that, contrary to cationic photosensitizing dyes, the singlet excited state of flavin molecules is not quenched by melanin.

- Motohashi N, Nishikawa H, Mori I.
Inhibitory effects of sulfur compounds on melanin formation reaction by tyrosinase. Chem Pharm Bull 39:142-145, 1991.
Abstract : The inhibitory effects of sulfur compds., namely cysteine and .alpha.-mercaptopropionylglycine (.alpha.-MPG) (thiol type), and N-methyl-2-mercaptoimidazole (MMI), 6-propylthiouracil (PTU), and ergothioneine (ESH) (thione type) on melanin formation reaction by tyrosinase, in the presence of tyrosine as a substrate, were assessed. Tyrosine oxidn. was monitored both by radioassay using L-[3,5-3H]tyrosine, L-[2,6-3H]tyrosine, and L-[carboxyl-14C]tyrosine as substrates, and by spectrometry to quantitate the dopachrome formed from L-tyrosine. The rated compds., ranked as to inhibitory effect in descending order are: cysteine .simeq. .alpha.-MPG > MMI > PTU > ESH. Cysteine and .alpha.-MPG, both noncyclic thiol compds., formed a 3,4-dihydroxyphenylalanine (dopa) conjugate to inhibit melanin formation when added at low concns. At high concns., they inactivated the enzyme by interacting with tyrosinase. In MMI, PTU, and ESH of heterocyclic thione structure, dopa conjugate formation was more inhibitory than tyrosinase inactivation on melanin formation.

- Napolitano A.
2-Aryl-1,3-thiazolidines as masked sulfhydryl agents for inhibition of melanogenesis. Biochim Biophys Acta 1073:416-422, 1991.
Abstract : As a part of an ongoing project aimed at developing new skin depigmenting agents, the ability of variously substituted 2-aryl-1,3-thiazolidines to inhibit melanogenesis in vitro was investigated. At 0.2 mM concentration 2-(2'-hydroxyphenyl)-1,3-thiazolidine-4-carboxylic acid (Th2), as well as the descarboxy analog (Th1) and, to a lower extent, the 4'-hydroxy isomer (Th3) all proved capable of preventing the tyrosinase catalyzed conversion of 0.2 mM L-tyrosine to melanin. Spectrophotometric monitoring of the reaction course in the presence of Th2 showed the initial formation of a yellow chromophore (lambda max 400 nm) which slowly decayed, being eventually replaced by a new absorption maximum centered at 305 nm. HPLC analysis of the final incubation mixture revealed the presence of a major product (lambda max 306 nm), ninhydrin and ferric chloride positive, which was isolated by gel filtration on Sephadex G-10 and was identified as beta-

[7-(3-carboxy-5-hydroxy-3,4-dihydro-2H-1,4-benzothiazinyl)]alanine (DBA) by 1H-NMR spectroscopy. Attempts to isolate the intermediate with lambda max 400 nm were hampered by its marked instability under the usual chromatographic conditions. However, the nature of the chromophore, coupled with mechanistic considerations, suggested for the compound the Schiff base-containing structure 3,4-dihydroxy-5-S-(N-salicylidenecysteinyl)phenylalanine (salcysdopa). This was substantiated by: (i) the formation of a zinc complex (lambda max 349 nm) analogous to that observed with the model Schiff base N-salicylidene leucine; and (ii) detection by 1H-NMR of a Schiff base resonance at delta 8.1 during the yellow chromophoric phase of the reaction. It was concluded that 1,3-thiazolidines inhibit melanin formation by a mechanism that involves the trapping of enzymically generated dopaquinone by the -SH containing Schiff base arising by cleavage of the thiazolidine ring. The salcysdopa adduct thus formed undergoes hydrolysis and subsequent ring closure to give eventually the colorless DBA.

- Paim S, Linhares LF, Mangrich AS, Martin JP.
Characterization of fungal melanins and soil humic acids by chemical analysis and infrared spectroscopy. Biol Fertil Soils 10:72-76, 1990.
Abstract : Humic acids from two Brazilian topsoils under savanna grassland and five soil fungal melanins were characterized by elemental, functional group and IR anal. C, N, total acidity, COOH, and phenolic OH contents were within the ranges reported for several other fungal melanins and soil humic acids. Compared with the soil humic acids, the IR spectra of the fungal melanins showed greater detail, indicative of higher aliphaticity. They were similar to the type III IR spectra of humic acids, which are characteristically high in proteinaceous material and polysaccharides. The IR spectra of the humic acids from the two Brazilian soils studied were classified as type I, which includes most soil humic acids. Notwithstanding the greater detail, in some areas the fungal melanin spectra were similar to those reported for other fungal melanins and humic acids of different origins. The probable contribution of the melanic fungi to the formation of soil humic polymers is discussed.

- Palumbo A.
Selective uptake of 2-thiouracil into melanin-producing systems depends on chemical binding to enzymically generated dopaquinone. Biochim Biophys Acta 1036:221-227, 1990.
Abstract : 2-Thiouracil (TU), an antithyroid drug, is receiving growing interest as a specific tumor marker for malignant melanoma, owing to its capability of being selectively accumulated into active melanin-producing tissues. However, up until now, the molecular mechanism of TU uptake by growing melanin has remained largely unknown. In an attempt to fill this gap, we have investigated the effect of TU on the tyrosinase catalyzed oxidation of tyrosine. At a concentration of 0.5 mM, TU was found to totally inhibit melanin formation by tyrosinase catalyzed oxidation of 0.25 mM tyrosine in phosphate buffer at pH 6.8. Polarographical monitoring of oxygen consumption under conditions of complete suppression of melanogenesis revealed a significant tyrosinase activity, with TU acting as a modest non-competitive inhibitor of the enzyme (Ki = 0.6 mM). HPLC and TLC analysis of the tyrosine-tyrosinase reaction in the presence of excess TU showed that the substrate is progressively consumed and a major hitherto unknown product (lambda max = 284 nm), positive to ninhydrin and ferric chloride, is concomitantly formed. This was isolated by repeated gel filtration chromatography of the reaction mixture on Sephadex G-10 and was formulated as the TU-dopa adduct 3,4-dihydroxy-6-(4'-hydroxypyrimidinyl-2'-thio)phenylalanine by spectral analysis. These results suggest that selective TU incorporation in pigmented melanomas and other melanin-producing systems is due to the covalent binding to dopaquinone, produced by tyrosinase catalyzed oxidation of tyrosine.

- Palumbo A.
Mechanism of inhibition of melanogenesis by hydroquinone. Biochim Biophys Acta 1073:85-90, 1991.
Abstract : Hydroquinone (HQ) is one of the most effective inhibitors of melanogenesis in vitro and in vivo, and is widely used for the treatment of melanosis and other hyperpigmentary disorders. In an attempt to get some insight into the molecular mechanism of the depigmenting action, which is still very poorly understood, we have investigated the effect of HQ on the tyrosinase catalysed conversion of tyrosine to melanin. Incubation of 0.5 mM tyrosine with 0.07 U/ml tyrosinase in phosphate buffer at pH 6.8 in the presence of 0.5 mM HQ led to no detectable melanin formation, due to the preferential oxidation of HQ with respect to tyrosine (HPLC evidence). Kinetic investigations showed that HQ is a poorer substrate of tyrosinase than

tyrosine; yet, it may be effectively oxidised in the presence of tyrosine owing to the generation of catalytic amounts of dopa acting as cofactor of tyrosinase. Product analysis of HQ oxidation with tyrosinase in the presence of dopa showed the predominant formation in the early stages of hydroxybenzoquinone (HBQ), arising from enzymic hydroxylation and subsequent oxidation of HQ, along with lower amounts of benzoquinone (BQ). These results suggest that the depigmenting activity of HQ may partly be related to the ability of the compound to act as an alternate substrate of tyrosinase, thereby competing for tyrosine oxidation in active melanocytes.

- Porebska-Budny M, Stepien K.
The effect of melanins on oxidation of lecithin in liposomal membranes. Acta Biochim Pol 37:89-92, 1990.
Abstract : Lecithin peroxidation in liposomal membranes induced by UV light was studied in the presence of natural eye melanin and synthetic melanins prepared from various precursors. It was shown that melanins inhibited lecithin photooxidation, and that the extent of this effect strongly depended on the type and concentration of melanin. Comparative study indicated that melanin obtained from adrenolutin was the most effective antioxidant. The ability to inhibit lipid peroxidation depends both on the concentration of paramagnetic centers in the melanin polymer and the accessibility of these centers for free radicals formed during irradiation of liposomes.

- Rodriguez-Lopez JN, Tudela J, Varon R, Garcia-Canovas F.
Kinetic study on the effect of pH on the melanin biosynthesis pathway. Biochim Biophys Acta 1076:379-386, 1991.
Abstract : This paper deals with the quantitative description of the regulatory effect of pH on the oxidation pathway of L-dopa to yield melanins. Tyrosinase catalyzes the oxidation by molecular oxygen of L-dopa to o-dopaquinone, which evolves non-enzymatically through a branched pathway with cyclization or hydroxylation reactions. The production of several quinones and semiquinones in the pathway has also been reported. The intermediates of the hydroxylation branch have been identified and the corresponding rate constants have been determined. These compounds, such as have been detected in melanosomes and in tumoral cells, have great cytotoxic power and could have physiological significance in acidic media.

- Rzepecki LM, Waite JH.
alpha,beta-Dehydro-3,4-dihydroxyphenylalanine derivatives: rate and mechanism of formation. Arch Biochem Biophys 285:27-36, 1991.
Abstract : The amino acid L-3,4-dihydroxyphenylalanine (DOPA), when present in the primary sequence of proteins, does not form melanin upon oxidation to the quinone, since its amine moiety participates in a peptide bond and cannot undergo internal cyclization. Instead, peptidyl DOPA quinone is available for other reactions. We have investigated the oxidation chemistry of a low molecular weight peptidyl DOPA analog, N-acetylDOPA ethyl ester (NAcDEE), and have shown that a major product of oxidation is an unsaturated DOPA derivative, N-acetyl-alpha,beta-dehydroDOPA ethyl ester (NAc delta DEE) (see companion paper, Rzepecki et al., Arch Biochem Biophys (1991) 285, 17-26). In the present study, we have explored kinetic and mechanistic features of the conversion of NAcDEE to NAc delta DEE and found that the reaction requires: (i) oxidation of NAcDEE to the quinone, (ii) the presence of a Lewis base as a catalyst (phosphate anion was the best of those tried in the pH range 6.0-8.0), and (iii) prevention of competing reactions such as Michael additions. Conversion efficiencies in the presence of Lewis bases ranged between 12 and 19% at pH 8.0 and 35 and 90% at pH 6.0. At least two separate reaction mechanisms appeared necessary to explain the kinetic data: (i) a pseudo-first-order mechanism at pH 6.0 and above, and (ii) an additional second-order mechanism at higher pH which involved both NAcDEE catechol and quinone. The apparent pseudo-first-order rate constants increased with pH from 2.36 X 10(-4) s-1 at pH 6.0 to about 30 X 10(-4) s-1 at pH 8.0 in 0.1 M sodium phosphate. Tautomerization of DOPA quinone to dehydroDOPA may thus be a factor in the sclerotization of natural structures incorporating DOPA containing proteins.

- Simonovic B, Vucelic V, Hadzi-Pavlovic A, Stepien K, Wilczok T, Vucelic D.
Thermogravimetry and differential scanning calorimetry of natural and synthetic melanins. J Therm Anal 36:2475-2482, 1990.
Abstract : The thermal stability of natural melanins from bovine eyes, black human hair and the hard core of banana peel, synthetic melanins obtained enzymically or by autoxidn. of various precursors, and chem.

modified synthetic melanins was studied by DSC and TG anal. It was shown that the resistance of melanins to thermal degrdn. depends on their origin. Synthetic melanins were more stable to thermal decompn. than natural melanins. Methylation of melanins caused a significant increase in thermal stability. The DSC curves of melanins reveal typical relaxation phenomena in the temp. range 293-413 K.

- Singh S, Dryhurst G.
Reactions of the serotonergic neurotoxin 5,6-dihydroxytryptamine with glutathione. J Org Chem 56:1767-1773, 1991.
Abstract : At physiol. pH the serotonergic neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) catalyzes the oxidn. of the cellular antioxidant GSH by mol. oxygen to give GSSG. At the stage when GSH is depleted, 5,6-DHT is then autoxidized to give first 2,7'-bis(5,6-dihyroxytryptamine) and ultimately indolic melanin. In the presence of an excess of GSH and enzymes such as tyrosinase or ceruloplasmin, the oxidn. of 5,6-DHT to its corresponding o-quinone (I) is greatly accelerated. Under such conditions, I is attacked by GSH to give 4-S-glutathionyl-5,6-dihydroxytryptamine (II), which is further oxidized to the corresponding quinone (III). Further attack by GSH or II on III gives 4,7-diglutathionyl-5,6-dihydroxytryptamine (IV) and 4,4'-di-S-glutathionyl-2,7'-bis(5,6-dihydroxytryptamine), resp. Reaction between III and IV yields 4,7,4'-tri-S-glutathionyl-2,7'-bis(5,6-dihydroxytryptamine).

- Sugumaran M, Semensi V.
Quinone methide as a new intermediate in eumelanin biosynthesis. J Biol Chem 266:6073-6078, 1991.
Abstract : The conversion of dopachrome to dihydroxyindole(s), a key reaction in eumelanin biosynthetic pathway, has been shown to be under the control of dopachrome conversion factor. Dopachrome conversion factor isolated from the hemolymph of Manduca sexta larvae, which is devoid of any tyrosinase activity, exhibits a narrow substrate specificity and readily bleaches the iminochromes derived from the oxidation of L-dopa, L-dopa methyl ester, and alpha-methyl-L-dopa, but failed to attack the corresponding D-isomers. The product formed in the case of L-dopachrome was identified to be 5,6-dihydroxyindole. Therefore, aromatization of dopachrome seems to accompany its decarboxylation as well. However, the enzyme also converts L-dopachrome methyl ester to an indole derivative indicating that it can deprotonate the alpha-hydrogen when the carboxyl group is blocked. These results are accounted for by the transient formation and further transformation of a reactive quinone methide intermediate during the dopachrome conversion factor-catalyzed reaction. The fact that the enzyme-catalyzed conversion of alpha-methyl dopachrome methyl ester (where both decarboxylation and deprotonation are blocked) resulted in the generation of a stable quinone methide in the reaction mixture confirms this contention and supports our recent proposal that quinone methide and not indolenine is the key transient intermediate in the conversion of dopachrome to dihydroxyindole observed during melanogenesis.

2. Biology of pigment cells and pigmentary disorders

- Aliev G, Rachkovsky M, Ito S, Wakamatsu K, Ivanov A.
Pigment types in selected color genotypes of Asiatic sheep. Pigment Cell Res 3:177-180, 1990.
Abstract : The types and amounts of pigments in fibers from variously colored Tajik, Hissar, and Caracul sheep were determined by three methods: high-performance liquid chromatography, electron spin resonance spectroscopy, and light microscopic evaluation of melanosomes. In both dominant and recessive black lambs the color is due to eumelanin pigment. Brown and red phenotypes are the result of interaction of AWt and EBl, EBr, or EY alleles, and these colors are caused by mixtures of eumelanin and pheomelanin in varying ratios. The HPLC and ESR measurements detected these differences in melanin type, while direct characterization of melanosomes generally failed to distinguish between melanin type or relative ratio of melanin type.

- Amir E, Gorsky M, Buchner A, Sarnat H, Gat H.
Physiologic pigmentation of the oral mucosa in Israeli children. Oral Surg Oral Med Oral Pathol 71:396-398, 1991.
Abstract : Physiologic melanin pigmentation (racial pigmentation) of the oral mucosa varies in prevalence among different races and ethnic groups. The purpose of this study was to investigate the prevalence of

physiologic pigmentation in Israeli Jewish children of different ethnic origins. A total of 1,300 children, 6 to 10 years of age, was examined. Physiologic pigmentation was found in 13.5% of the population studied. Children of Eastern origin showed a significantly higher prevalence of pigmentation compared with Ashkenazi and Sephardic groups. Because melanin pigmentation can be enhanced by mechanical and chemical stimulation (smoking), this study may serve as a baseline for investigation of melanin pigmentation in various ethnic groups.

- Ando H, Oka M, Ichihashi M, Mishima Y.
Protein kinase C and linoleic acid-induced inhibition of melanogenesis. Pigment Cell Res 3:200-206, 1990.
Abstract : Linoleic acid has been shown to inhibit melanogenesis in cultured B16 mouse melanoma cells. We report here the possible involvement of protein kinase C (PKC) in linoleic acid-induced inhibition of melanogenesis in B16 cells. A single PKC subspecies (alpha-PKC) was detected in B16 cells. The enzyme was activated by linoleic acid in vitro. The effective concentrations at which PKC was activated (25 microM; maximum response) were consistent with those for the inhibition of melanogenesis in cell culture system. In addition, the permeable diacylglycerol 1-oleoyl-2-acetyl glycerol that activates PKC also inhibits melanogenesis at 100 microM. These results suggest that activation of PKC plays a pivotal role in the linoleic acid-induced inhibition of melanogenesis in B16 cells.

- Balkema GW, Drager UC.
Origins of uncrossed retinofugal projections in normal + and hypopigmented mice. Vis Neurosci 4:595-604, 1990.
Abstract : In albinos, the retinofugal projections to the ipsilateral side of the brain are reduced (e.g., see Guillery, 1969; La Vail et al., 1978; Lund, 1965). Although all ganglion cell types are affected, in mice the displaced ganglion cell population is the main target of the albino mutation (Drager & Olsen, 1980). Here we tested whether this preferential effect on displaced ganglion cells is a general consequence of the melanin reduction or a pleiotropic effect unique to the albino locus, by retrogradely tracing retinal ganglion cells in normal C57BL/6J mice and in several non-allelic hypopigmentation mutants on the same background: albino (C57BL/6J-c2J), beige (C57BL/6J-bg), pale ear (C57BL/6J-ep), ruby-eye/haze (C57BL/6J-ru-2hz), and pearl (C57BL/6J-pe). All mutants have lower overall cell counts in the ipsilateral projection, but the displaced population is disproportionately affected: the albinos contain 42% of the normal number of displaced ganglion cells, and the other mutants have an average 57% of normal counts. The reduction in uncrossed retinofugal projections in albinos affects the inputs to the lateral geniculate nucleus and the superior colliculus, but not to the suprachiasmatic nucleus (Drager, 1974). To address the question in which way the susceptible uncrossed projections differ from the nonsusceptible one, we compared ganglion cells backfilled from the suprachiasmatic nucleus to ganglion cells backfilled from the optic tract at geniculate level. Whereas the uncrossed optic tract projection originates from the binocular region in the ventro-temporal retina and contains a high fraction of large and displaced ganglion cells (Drager & Olsen, 1980), both the crossed and uncrossed inputs to the suprachiasmatic nucleus originate from the entire retina with a relative preference for the lower nasal region that corresponds to part of the monocular visual field; all ganglion cells projecting to the suprachiasmatic nucleus are of medium size, and they are located in the ganglion cell layer. These observations allow the following conclusions: (1) All genetic mutants which cause a reduction in ocular melanin, regardless of the molecular or cell-biological mechanism underlying the pigment reduction, result in decreased uncrossed projections; this confirms previous reports (La Vail et al., 1978, Sanderson et al., 1974). (2) The decrease affects only projections involved in binocular vision. (3) In mice, the ganglion cells displaced to the inner nuclear layer, and hence located closer to the retinal pigment epithelium, are disproportionately affected by the melanin reductions. These observations may provide cues to the spatio-temporal mechanism of the

- Bechtel HB.
Inherited color defects. Comparison between humans and snakes. Int J Dermatol 30:243-246, 1991.

- Berardesca E, de Rigal J, Leveque JL, Maibach HI.
In vivo biophysical characterization of skin physiological differences in races. Dermatologica 182:89-93, 1991.
Abstract : The role of race in modulating skin responses has been investigated. Several parameters (skin thickness, transepidermal water loss, water content of the stratum corneum and skin biomechanics) have

been measured using noninvasive tools in whites, Hispanics and blacks to assess whether the melanin content could induce changes in skin biophysical properties. Marked differences between races appear in stratum corneum water content and in skin extensibility, recovery and elastic modulus. Measurements done in different sun-exposed sites highlight the effects of solar irradiation on the skin and the role of melanin in preventing skin damage. The study shows that racial differences in skin physiology exist and are mainly related to the protective role of melanin present in races with darker skin. Moreover, differences in skin hydration are not fully explained according to the site and presence of hair.

- Campo JL, Alvarez C.
Further study on the plumage pattern of the Blue Andalusian breed. Poult Sci 70:1-5, 1991.
Abstract : The genetic basis for plumage color of the Blue Andalusian breed was studied. Results of crosses between Blue Andalusian females and Brown (eb/eb) tester males showed that this genetic stock was E/E and did not carry a columbian-type gene. This fact was further verified by the cross between Blue Andalusian males and Melanotic Prat (eWh/eWh Co/Co Ml/Ml) females. It is suggested that the Bl/bl+ genotype is effective in changing black to blue pigment when only one eumelanizing gene is present in the genetic background, but it is ineffective in the presence of two different genes producing eumelanin simultaneously. With an E/E genotype, Bl/bl+ does not change black pigment to blue in the areas where the melanotic (Ml) or lacing (Lg) genes produce black pigment, resulting in the laced plumage pattern of the Blue Andalusian (E/E Bl/bl+ Ml-Lg/Ml-Lg). On a non-E/E genetic background, a single dosage of Bl changes the black pigment to blue in the presence of the melanotic or lacing genes. Double-laced phenotypes were not found in either cross, thereby causing the authors to question the role of Co in double and single-laced patterns. Linkage between Ml and Lg was estimated to be 12.2% +/- 2.1 (SE).

- Dzhavakhiya VG, Aver'yanov AA, Minaev VI, Ermolinskii BS, Voinova TM, Lapikova VP, Petelina GG, Vavilova NA.
Structure and function of cell wall melanin of Pyricularia oryzae cav, the rice blast causative agent. Zh Obshch Biol 51:528-535, 1990.
Abstract : Anal. of P. oryzae mutants with mycelium color disorders showed that pentaketide melanin in the outer layer of the cell wall is the fungal pigment. Genetic blocking of melanin formation decreased infectivity of the conidia by 2 orders of magnitude. Melanin decreased the lethal effect of light, protected the fungus from O2 generated by rice leaves, and also protected the cell wall of the fungus from the hydrolytic enzymes of the host.

- Fujita H, Orita Y, Iio K.
Relationship between melanin in the iris and in the stria vascularis. Ear Res Jpn 21:91-92, 1990.
Abstract : The amt. of melanin in the stria vascularis was computed, using three types of hamsters different in coat and iris color. The amt. of melanin contained in the intermediate cells correlated with the degree of pigmentation in the animal's coat and eyes. Wild type hamsters were found to contain dendritic melanocytes not found in TPB on MP. Thus, the melanocyte of the inner ear is controlled by genotype P. The amt. of melanin in stria vascularis tends to increase in proportion to the amt. of the melanin in iris.

- Gadd GM, Gray DJ, Newby PJ.
Role of melanin in fungal biosorption of tributyltin chloride. Appl Microbiol Biotechnol 34:116-121, 1990.
Abstract : Intact biomass of an albino and a melanic strain of Aureobasidium pullulans, as well as purified melanin from the latter strain, was capable of Bu3SnCl removal from soln. Melanized biomass had a greater biosorptive capacity than albino biomass, this difference being attributable to the presence of melanin. Purified melanin had a large capacity for Bu3SnCl biosorption, the calcd. max. uptake capacity being .apprx.35 mmol/g dry wt. Bu3SnCl biosorption by intact biomass and melanin obeyed the Langmuir adsorption isotherm over the concn. range used, and was relatively unaffected by pH 3.5-6.5; .apprx.20% decrease in Bu3SnCl biosorption resulted at pH 2.5. A Bu3SnCl concn. of 0.3 .mu.M in growth medium resulted in a lag period which was longer with the albino strain (.apprx.50 h) than with the pigmented strain (.apprx.25 h). The addn. of melanin to Bu3SnCl-contg. growth media resulted in a redn. in toxicity and attainment of higher cell yields. The applied and environmental significance of these interactions are discussed.

- Gochfeld M, Saliva J, Lesser F, Shukla T, Bertrand D, Burger J.
Effects of color on cadmium and lead levels in avian contour feathers. Arch Environ Contam Toxicol 20:523-526, 1991.
Abstract : The use of feathers has been proposed as a noninvasive tissue for biomonitoring metal levels in birds and their ecosystems. The authors examd. cadmium and lead levels in black and white contour feathers from common (Sterna hirundo) and sooty (S. fuscata) terns and black skimmers (Rynchops niger) to det. if there were significant differences in metal levels related to the melanin content of the feathers. There were no significant differences in metals in black vs. white body feathers in any of the three species; correlations between metal residues from individual birds were low.

- Gonzalez-Campora R, Armas-Padron JR, Rios-Martin JJ, Lopez-Garrido J, Gomez-Pascual A, Galera-Davidson H.
Nucleolar organizer regions in pigmented skin lesions. Value in the differential diagnosis of Spitz nevi. Anal Quant Cytol Histol 13:16-22, 1991.
Abstract : Forty-one cases of typical melanocytic skin lesions (15 intradermal nevi, 14 Spitz nevi and 12 malignant melanomas) were used to investigate the value of staining of nucleolar organizer regions (NORs) in the differential diagnosis of such pigmented lesions. Histologic sections were stained by the silver colloid (Ag) method, with and without the prior use of a melanin blocking agent. There were statistically significant differences in the mean numbers of AgNORs per nucleus between the groups of lesions studied (1.658 for intradermal nevi, 3.0042 for Spitz nevi and 6.669 for malignant melanomas). Sections treated with potassium permanganate (melanin blocking agent) prior to staining showed an obvious increase in the AgNOR scores in all groups; this increase was highest for Spitz nevi. Although AgNOR staining allows a distinction to be made between intradermal nevi and malignant melanomas, the striking overlap between the counts for Spitz nevi and malignant melanomas precludes the use of this technique as the sole method for establishing the diagnosis of malignancy. Other clinical and morphologic data are especially required to make the diagnosis of Spitz nevi.

- Hedin CA, Axell T.
Oral melanin pigmentation in 467 Thai and Malaysian people with special emphasis on smoker's melanosis. J Oral Pathol Med 20:8-12, 1991.
Abstract : At the faculties of dentistry in Chiang Mai, Thailand (CM), and Kuala Lumpur, Malaysia (KL), 234 and 233 consecutive out-patients were interviewed concerning tobacco and chewing habits and examined for the presence of oral melanin pigmentation. Tobacco was regularly used by 32% and 28% of the studied populations in CM and KL. Cigarette smoking was the predominant habit, but the chewing of betel and tea leaves (miang) and the smoking of banana leaf cigars (khi yo) was also registered. The genetically acquired pigmentation dominated. Although nearly all non-tobacco users in the Malay and Indian populations had oral melanin pigmentation, it was found that tobacco smokers had significantly more oral surfaces pigmented than non-tobacco users. Among Thais, the percentage of pigmented individuals was significantly higher among tobacco smokers. It was concluded that tobacco smoking stimulates oral melanocytes to a higher melanin production also in dark-skinned ethnic groups.

- Hill SE, Bleehen SS, MacNeil S.
1.alpha.-25-Dihydroxyvitamin D3 increases intracellular free calcium in murine B16 melanoma. Br J Dermatol 120:21-30, 1989.
Abstract : Vitamin D3 and its active metabolite 1.alpha.-25-dihydroxyvitamin D3 (1.alpha.-25-(OH)2D3) have been reported to play a role in melanogenesis. Physiol. concns. of 1.alpha.-25-(OH)2D3 acutely elevated intracellular free Ca (using Fura 2) in BI6 primary (I.degree.) cells. Membrane phosphoinositide turnover was unaffected by 1.alpha.-25-(OH)2D3. The rise in intracellular free Ca was entirely dependent on extracellular Ca and was not mimicked by vitamin D3. However, in neither BI6-I.degree. nor BI6-F1 melanoma cells did vitamin D3 or 1.alpha.-25-(OH)2D3 increase melanin prodn.

- Horikoshi T, Balin AK, Carter DM.
Effects of oxygen tension on the growth and pigmentation of normal human melanocytes. J Invest Dermatol 96:841-844, 1991.
Abstract : The effects of oxygen tension on human melanocyte growth, tyrosinase activity, and melanin

production were assessed. Melanocytes, seeded at 10(4) cells/cm2, were grown in modified Eagle's medium (MEM) with 5% fetal bovine serum (FBS) and 10 ng/ml 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Flasks were equilibrated with gas mixtures containing 5% CO2 and various partial pressures of oxygen (PO2 7-620 mm Hg) and kept in incubators, which were electronically maintained at the desired oxygen tensions. Melanocytes grew best at PO2 from 6-34 mm Hg. Growth was reduced by 30% at PO2 142 mm Hg, and even more at O2 tensions greater than 230 mm Hg. A PO2 of 603 mm Hg was cytotoxic. Tyrosinase activity (assayed by the method of Pomerantz) was 300 microU/mg protein at PO2 7-34 mm Hg. At PO2 235 and 355 mm Hg tyrosinase activity decreased to about 100 microU/mg protein. The apparent Km for tyrosine was unchanged in melanocytes cultured at all experimental oxygen tensions. The Vmax, however, was decreased at the higher oxygen tensions (PO2 235 mm Hg). At PO2 6-135 mm Hg the melanin content was proportional to tyrosinase activity. At cytostatic oxygen tensions (PO2 235 and 355 mm Hg) the intracellular melanin content increased somewhat, although tyrosinase activity was decreased. Low oxygen tension is favorable for both melanocyte proliferation and tyrosinase activity.

- Howe J, Costantino R, Slominski A.
On the putative mechanism of induction and regulation of melanogenesis by L-tyrosine. Acta Derm Venereol (Stockh) 71:150-152, 1991.
Abstract : The stimulation of melanogenesis by L-tyrosine in hamster melanoma is several-fold higher than that by norepinephrine, epinephrine, clonidine and isoproterenol and absent in the case of tyramine dopamine and phenylephrine. Therefore, the melanogenic effect of L-tyrosine in hamster melanoma follows a different pathway than that linked to the activation of dopaminergic and adrenergic receptors.

- Ishihara Y, Oka M, Tsunakawa M, Tomita K, Hatori M, Yamamoto H, Kamei H, Miyaki T, Konishi M, Oki T.
Melanostatin, a new melanin synthesis inhibitor. Production, isolation, chemical properties, structure and biological activity. J Antibiot (Tokyo) 44:25-32, 1991.
Abstract : Melanostatin, a new antibiotic with melanin synthesis inhibitor activity, was isolated from the fermentation broth of Streptomyces clavifer No. N924-2. Its structure was determined by spectral analysis and degradation experiments. Melanostatin strongly inhibited melanin formation in Streptomyces bikiniensis NRRL B-1049 and B16 melanoma cells.

- Kaufmann D, Wiandt S, Veser J, Krone W.
Increased melanogenesis in cultured epidermal melanocytes from patients with neurofibromatosis 1 (NF 1). Hum Genet 87:144-150, 1991.
Abstract : Melanocyte cultures from the normally pigmented skin of patients with neurofibromatosis 1 (NF 1) have a higher melanin content than those from the skin of healthy donors. An additional increase in the amount of melanin per cell was found in 5 out of 6 lines of melanocytes derived from cafe au lait macules of NF 1 patients. Omission of the tumor promoter phorbol-12-myristate-13-acetate from the culture medium brings about a comparable increase in the melanin content in all three kinds of melanocyte cultures. Cultures of NF 1 melanocytes show a higher tyrosine hydroxylase activity than those of control melanocytes, and incorporate larger amounts of dihydroxyphenylalanine than the latter. We conclude that melanogenesis in epidermis melanocytes is affected by defective alleles of the NF 1 gene. Our findings do not contradict the hypothesis that the defect underlying NF 1 impairs the inhibition of a wild-type RAS oncogene by interfering with the GTPase-activating function of the NF 1 gene product.

- Kita T, Hayashiba Y, Ohshima T, Minoda M, Tanaka N.
Determining aging changes of melanin granules of human scalp hairs by image analyser. Nippon Hoigaku Zasshi 45:44-51, 1991.
Abstract : Using electron micrographs of human hairs, we measured the minor axis and density of hair melanin granules in a 0 year old infant, 20-30 year old adult and 60-70 year old adult by an image analyser for the purpose of determining the aging changes of melanin granules of human scalp hairs. The melanin density of the outer hair cortex was higher than that of the inner hair cortex. There were no differences between the 0 year old infant and the 20-30 year old adult in the minor axis and density of melanin granules, but significant differences were evident in the minor axis between the 60-70 year old adult and the other generations. Significant differences were noted in the density of melanin granules of the inner hair cortex between the 60-70 year old adult and the other generations. As a result of the quantitative analyses,

it was proved that the melanin granules of human scalp hair increase in size and decrease in number by age.

- Koch PB, Nijhout HF.
Color pattern specific proteins in black scales in developing wings of Precis coenia Huebner (Nymphalidae, Lepidoptera). Roux's Arch Dev Biol 199:289-294, 1990.
Abstract : A set of stage specific proteins of approx. 86 to 90 kDal are synthesized by isolated wings of P. coenia on day 5 of the pupal stage. They are named B proteins. They are synthesized in presumptive black wing areas in higher amts. than in presumptive white wing areas and are the major proteins synthesized on day 5. Wings from 5 day old pupae, which were incubated with 35S-methionine for 2 or 4 h, incorporate radiolabel into presumptive black pattern elements. This is probably due to the localized synthesis of the above mentioned proteins. Injection of 35S-methionine into whole pupae on day 5 resulted in the labeling of the mature black and grey scales but not white scales. This radiolabel incorporation pattern corresponds exactly to the incorporation of the melanin precursor 14C-tyrosine into the scales. The results indicate that the B proteins are specifically related to the formation of black and grey portions of the Precis wing pattern. Injection of 35S-methionine into whole pupae on day 6 resulted in the labeling of the mature red scales probably due to labeling of R proteins, which may be involved in the formation of red pattern elements.

- Lamyai H, Vanichjakvong O.
Gingival melanotic macule: a case report. J Dent Assoc Thai 40:1-7, 1990.
Abstract : This case report deals with 17-year old female who was diagnosed as having gingival melanotic macule at the gingiva of the tooth number 21, 22 and 23 on labial and palatal aspects. Gingival melanotic macule is pathological condition of melanin pigment accumulation or melanin pigment production. The black area was enlarging, although the gingival architecture was in physiologic condition. It was treated by the method of free autogenous gingival graft on the labial side and gingivectomy by flap on the palatal side.

- Martinez G, Carnazza M.
Melanosomes, melanocytes and keratinocytes in the human epidermis in incontinentia pigmenti. Arch Ital Anat Embriol 95:65-76, 1990.
Abstract : A functioning epidermal melanin unit implies a melanocyte capable of transferring melanosomes to keratinocytes; this requires not only melanocytes with adequate dendrites but also "receptive" keratinocytes. Skin with incontinentia pigmenti was examined by electron microscopy. Premelanosomes were occasionally found within keratinocytes and deposits of extracellular granular material that came from vacuolar degeneration of keratinocytes adjacent to melanocytes.

- Matsuoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW.
Racial pigmentation and the cutaneous synthesis of vitamin D. Arch Dermatol 127:536-538, 1991.
Abstract : The varying epidermal melanin content that produces racial pigmentation determines the number of photons that reach the lower (malpighian) cellular layers, where vitamin D3 synthesis takes place. We investigated the effect of racial pigmentation on vitamin D3 formation, stimulating the process with a fixed dose of UVB radiation (wavelengths, 290 to 320 nm). Vitamin D nutritional status was further assessed measuring serum 25-hydroxyvitamin D and the most active serum metabolite, 1,25-dihydroxyvitamin D. Experimental subjects were young (third decade of life) and healthy, representing the white, Oriental (East Asian), Indian (South Asian), and black races. Basal serum vitamin D3 levels were similar among groups, ranging from 2.3 +/- 0.6 nmol/L (mean +/- SEM) for blacks to 3.4 +/- 1.0 nmol/L for Indians. Following whole-body exposure to 27 mJ/cm2 of UVB, there was a significant racial group effect on serum vitamin D3 levels. Post-UVB levels were significantly higher in whites (31.4 +/- 4.4 nmol/L) than in Indians or blacks (12.8 +/- 2.9 and 9.1 +/- 2.1 nmol/L, respectively), while the levels in Orientals (27.8 +/- 4.4 nmol/L) differed significantly from those in blacks and Indians but not in whites. Race had only a marginal effect on serum 25-hydroxyvitamin D, with higher levels in whites than in blacks (69.9 +/- 12.7 vs 29.7 +/- 6.2 nmol/L). Serum 1,25-dihydroxyvitamin D and vitamin D binding protein levels were similar in all groups. We conclude that while racial pigmentation has a photoprotective effect, it does not prevent the generation of normal levels of active vitamin D metabolites.

- Menon IA, Persad SD, Haberman HF, Basu PK, Norfray JF, Felix CC, Kalyanaraman B.
Characterization of the pigment from homogentisic acid and urine and tissue from an alkaptonuria patient.

Biochem Cell Biol 69:269-273, 1991.
Abstract : When urine samples from alkaptonuria patients are allowed to stand, they turn black, presumably owing to the oxidation of homogentisic acid to a melanin-like substance. We report the characterization of the pigments formed by polymerization of (a) the components in the urine from a patient with alkaptonuria and (b) homogentisic acid. The absorption spectra and electron spin resonance signals of these pigments are similar to those of eumelanins. Irradiation of the pigments with nitroblue tetrazolium caused reduction of the tetrazolium; this was partially inhibited by superoxide dismutase. Irradiation of Ehrlich ascites carcinoma cells with the pigments from homogentisic acid or urine caused cell lysis. Since this lysis was inhibited by catalase, we have concluded that it was mediated by H2O2. A similar pigment was also extracted from the tissue from an alkaptonuria patient. It is suggested that thedegeneration of tissue in vivo may be due to the deposition of melanin-like pigments in the tissues, probably in combination with metal ions.

- Miyashita Y, Moriya T.
Calcium ion in chromatic nerve transmission and melanophore movements in teleosts. J Exp Zool 256:121-129, 1990.
Abstract : Ca ionophore A 23187 was examd. for its action on innervated and denervated melanophores of the guppy Lebistes reticulatus and the Siamese fighting fish, Betta splendens. This Ca ionophore (5 .times. 10-67M) induced melanin aggregation in innervated melanophores of both species. This aggregating response disappeared in the presence of an .alpha.-adrenolytic agent, phentolamine. However, in denervated skin melanophores of these fish, A 23187 failed to aggregate melanosomes. An inhibitor for intracellular Ca release, TMB-8, inhibited the melanophore-aggregating responses induced by A 23187 and by elec. stimulation of nerves. In the presence of TMB-8 (<10-4M), the melanin aggregation response to norepinephrine (NE) commenced normally and both the rate and degree of aggregation were identical to those in saline soln. alone. However, the time course of re-dispersion from the aggregated state after NE treatment clearly shortened with TMB-8. This action of TMB-8 on the response to NE was enhanced in Ca-free saline soln. The possible roles and origin of Ca2+ in chromatic nerve transmission and melanophore movements in teleost fish are discussed.

- Morison WL, Kerker BJ, Tunnessen WW, Farmer ER.
Disseminated hypopigmented keratoses. Arch Dermatol 127:848-850, 1991.
Abstract : We present two cases of asymptomatic, widespread keratotic eruptions in young female patients. Clinically, the lesions are well-demarcated, small, hypopigmented, flat-topped papules occurring on the trunk and extremities in a uniform distribution. Skin biopsy specimens from one patient revealed hyperorthokeratosis, papillomatosis, and a normal amount of melanin. We suggest that this is a newly recognized dermatologic entity that may be descriptively termed disseminated hypopigmented keratoses. Disseminated hypopigmented keratoses may be distinguished by clinical and histologic criteria from similar keratotic eruptions. Since the lesions of disseminated hypopigmented keratoses are both inconspicuous and asymptomatic, it is likely that the disorder is more prevalent than our two cases would suggest.

- Ohkuma M.
Presence of melanophages in the normal Japanese skin. Am J Dermatopathol 13:32-37, 1991.
Abstract : Human skin samples were obtained from the normal peripheral portion of specimens removed from persons with various cutaneous and systemic diseases. A portion of each specimen was embedded in paraffin and another part in water-soluble embedding medium, and some was frozen in liquid nitrogen for light microscopy and histochemistry. Some specimens were also investigated by electron microscopy. In 31 of 32 specimens, cells containing brown pigment were observed in the superficial dermis. Because both acid phosphatase and Masson-Fontana staining were positive and the 3,4-dihydroxyphenylalanine reaction negative, the cells were considered to be melanophages. Electron microscopic examination revealed that these cells contained melanosome-laden phagosomes. Some fibroblastlike cells were also observed with intracellular single or multiple melanosomes. This study documents the occurrence of melanophages in the normal skin of Japanese subjects.

- Orlow SJ, Chakraborty AK, Boissy RE, Pawelek JM.
Inhibition of induced melanogenesis in Cloudman melanoma cells by four phenotypic modifiers. Exp Cell Res 191:209-218, 1990.

Abstract : Retinoic acid, hexamethylene bisacetamide, sodium butyrate, and dimethylsulfoxide, four compounds which modulate phenotypic expression in a variety of neoplastic cell lines, all inhibited the induction of tyrosinase activity and melanogenesis by the combination of melanocyte-stimulating hormone and isobutylmethyxanthine in Cloudman S91 melanoma cells. Results were the same in assays of whole cells or in extracts made from them. Only retinoic acid, however, was effective at inhibiting the activation of dopachrome isomerase, another regulatory enzyme in melanogenesis. Despite inhibiting the effects of melanocyte-stimulating hormone (MSH) and isobutylmethylxanthine on tyrosinase activity, all of the agents tested increased the binding of MSH to intact cells. Ultrastructural analysis of treated cells following DOPA cytochemistry revealed that both retinoic acid and hexamethylene bisacetamide arrested melanosomal maturation at stage I-II. Retinoic acid resulted in a derangement of melanosomal structure. The specificity of these agents for preventing the induction of melanogenesis makes them powerful tools for the dissection of this complex cellular process.

- Pearson CA.
Hypomelanosis in tuberculosis--unrelated to anaemia. East Afr Med J 67:419-426, 1990.
Abstract : Previous work has shown that hypomelanosis is a useful sign to aid suspicion of tuberculosis in patients with dark skins. This study has confirmed that it is unrelated to haemoglobin levels. A simple colour card 'melanometer' for clinical use is described. As hypomelanosis has not been noted in HIV infection in African, and as tuberculosis is an important differential in case-definition where diagnostic facilities are limited, it is suggested that more clinical research on hypomelanosis in relation to disease is urgent.

- Polak A.
Melanin as a virulence factor in pathogenic fungi. Mycoses 33:215-224, 1990.
Abstract : The pigment melanin is found universally in nature and is attributed to a variety of functions. In some fungi it is thought to play a decisive role in the determination of virulence. This review examines the experimental evidence which has led to an understanding of the mechanisms by which melanin functions in pathogenic fungi, particularly in plant pathogens, in Cryptococcus neoformans and Wangiella dermatitidis.

- Roth RI, Baker G, Levin J.
An animal model for the study of azidothymidine-induced hyperpigmentation. Lab Invest 64:437-439, 1991.
Abstract : Mice fed azidothymidine demonstrated dramatic hyperpigmentation of their tails. Histologic examination demonstrated large quantities of melanin pigment throughout the entire epidermal layer. Control mice had scant melanin pigment localized to the basal layer. Our findings demonstrate that the mouse is a useful model for the investigation of drug-induced hyperpigmentation.

- Saito H, Mukaiyama F.
Hormonal control of melanization in newly hatched larvae of the saturniid silkmoth, Samia cynthia ricini Donovan (Lepidoptera:Saturniidae). Appl Entomol Zool 25:323-325, 1990.
Abstract : The relationship between hormonal control and cuticular melanization of newly hatched larvae in the saturniid silkmoth, S. cynthia ricini, was investigated. The melanization of the 1st instar larvae in Samia is controlled by humoral factors, which are released from the head region rather than the thorax-abdominal region. Thus, it seems that MRCH-like substance controls melanization in newly hatched larvae of Samia.

- Saito N, Morishima T.
Eumelanin and pheomelanin contents in hair and 5-S-cysteinyldopa and 5-hydroxy-6-methoxyindole-2-carboxylic acid levels in urine in Japanese oculocutaneous albinism. Arch Dermatol Res 283:7-9, 1991.

- Sanz J.
Melanin in the prostate gland. Arch Esp Urol 43:780-782, 1990.
Abstract : We report two rare cases of prostatic melanosis incidentally discovered on pathological examination of specimens from a prostate biopsy (adenocarcinoma) and open surgery (benign hyperplasia). We describe the features and discuss the hypotheses relative to this disease entity.

- Schachtschabel DO, Selzer R.

Inhibition of growth and melanin formation in cultured melanoma cells using azelaic acid and other dicarboxylic acids. Martin-Luther-Univ., Halle-Wittenberg, 41, Malig. Melanom), 97-100, 1989.
Abstract : The effects of C4-C11 dicarboxylic acids on cell growth and melanin formation were studied in cultures of Harding-Passey melanoma. The inhibitory effects increased with the carbon chain length. The melanin formation was more sensitive to the inhibition than the tumor cell growth.

- Searle AG.
Comparative genetics of albinism. Ophthalmic Paediatr Genet 11:159-164, 1990.
Abstract : Albinism in laboratory mammals is equivalent to human tyrosinase-negative oculocutaneous albinism, and thus the result of recessive mutation in the structural locus for tyrosinase (TYR), which prevents melanin biosynthesis. In the mouse, eight mutant alleles are now known at this locus, with differing effects on eye colour and on the degree of reduction in eumelanin and phaeomelanin pigmentation. Three of these alleles, namely chinchilla, himalayan (acromelanistic) and albino (c) itself, have also been recognized in a number of other species but only albino has been identified in man so far. The himalayan allele (equivalent to Siamese in the cat) is of particular interest because it converts tyrosinase into a thermolabile form, with greater production of melanin in colder areas of the body. The optic track misrouting found in human albinos also occurs in albino alleles in other mammals, which may also show reduced activity and stress responses. The TYR locus is on human chromosome 11, which now has at least 11 loci with homologues on mouse 7. However, their order is markedly different in the two species. For instance, c and Hbb (beta-globin), which are closely linked in mouse, rabbit, cat etc., are far apart on human 11q and 11p respectively. Moreover, some loci (e.g., Fes and Mod-2) which are close to c in the mouse appear to be on human chromosomes other than 11. This extensive chromosomal restructuring in mammalian evolution means that the effects of human albino deletions may differ greatly from those studied in the mouse, which are associated with defects of kidney, liver and thymus. Tyrosinase-positive albinos or near-albinos are known at a number of loci in mice and other mammals. They are the result of the absence or inhibition of melanocytes in the affected areas, so that no melanin is produced. In general they are associated with pathological pleiotropisms which may lead to anaemia, inner ear defects, megacolon, neurological effects, skeletal defects, microphthalmia, osteopetrosis, spina bifida, sterility and so on. Homologies between these and human loci affecting pigmentation are now being discovered.

- Slominski A, Paus R, Costantino R.
Differential expression and activity of melanogenesis-related proteins during induced hair growth in mice. J Invest Dermatol 96:172-179, 1991.
Abstract : In C57 Bl-6 mice, the sequence of tyrosinase (key enzyme of melanogenesis) expression and activity and the presence of the melanosomal protein gp 75 were studied during the development of traumatically induced anagen follicles (day 0 = telogen, and days 1-12, after anagen induction studied). In addn. to performing Northern and Western blots for tyrosinase, tyrosine hydroxylase activity (THA) and dopa oxidase activity (DOA) were measured. On day 0, DOA was undetectable, and THA was very low. On days 1 and 2, both activities were undetectable; starting from day 3, they increased rapidly, reaching a plateau on days 8 and 12. DO-pos. proteins had apparent mol. wts. (MW) of 66-68 kD (days 3-12), 72-74 kD (days 5-12), and 130 kD (days 8 and 12). Western blotting emphasized proteins of MW 66-68 kD (tyrosinase), and 73-75 kD (gp 75); tyrosinase was undetectable on day 0, but already present on days 1 and 2; it increased by day 5 and had reached a plateau on days 8 and 12; gp 75 was undetectable on days 0-2; it was present on day 3, increased by day 5, and reached a plateau on days 8 and 12. Northern blot anal. revealed high levels of tyrosinase mRNA on days 5 and 8, low levels on days 1-3, and none on day 0. These data suggest a highly regulated, time frame-restricted, differential pattern of tyrosinase transcription, translation, and enzyme activity during the different stages of the developing murine anagen follicle, possibly as a result of complex interactions between follicular melanocytes and their environment.

- Sundberg JP.
Pigmented spleens in C57BL mice. Lab Anim 25:85-86, 1991.

- Swope VB, Abdel-Malek Z, Kassem LM, Nordlund JJ.
Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol 96:180-185, 1991.

Abstract : Interleukin (IL)-1 alpha, IL-6, and tumor necrosis factor (TNF)-alpha are epidermal cytokines that produce many similar biologic effects. We have investigated the possibility that these cytokines act as regulators of melanization and proliferation of cultured normal human melanocytes (NHM). All three cytokines elicited a dose-dependent decrease in the activity of the enzyme tyrosinase after 48 h of treatment. IL-1 alpha had the greatest inhibitory effect, resulting in a 22% inhibition of tyrosinase activity at a concentration of 3 x 10(-14) M. An equivalent effect was elicited by 4 x 10(-11) M IL-6 and 10(-11) M TNF-alpha. All three cytokines also inhibited melanocyte proliferation, as measured by a decrease in the rate of 3H-thymidine incorporation and an increase in doubling time. IL-1 alpha at 6 x 10(-14) M, 6 x 10(-13) M, and 3 x 10(-12) M, TNF-alpha at 10(-10) M, 10(-9) M, and 10(-8) M, and IL-6 at 4 x 10(-10) and 1.2 x 10(-9) M produced a dose-dependent inhibition of 3H-thymidine incorporation. The effects of IL-1 alpha, TNF-alpha, and IL-6 were cytostatic, not cytotoxic, because melanocytes remained viable following several treatments with the cytokines. Also, melanocytes treated with IL-1 alpha and TNF-alpha recovered and resumed proliferation after cessation of treatment. These effects of IL-1 alpha, IL-6 and TNF-alpha do not seem to be mediated by stimulation of eicosanoid production, because inhibition of arachidonic acid (AA) metabolism by indomethacin, a cyclooxygenase inhibitor, and nordihydroguaiaretic acid, a lipoxygenase inhibitor, did not reverse the inhibitory effects on either proliferation or tyrosinase activity of NHM. This is the first demonstration that NHM respond to epidermal cytokines, and suggests a role for paracrine and possibly autocrine regulation of melanocytes by immune modulators.

- Tobin DJ, Fenton DA, Kendall MD.
Ultrastructural study of exclamation-mark hair shafts in alopecia areata. J Cutan Pathol 17:348-354, 1990.
Abstract : The prime diagnostic feature of acute alopecia areata is the presence of exclamation mark hairs. These characteristic hairs fracture at their distal end and taper proximally towards the scalp, giving them the appearance of an exclamation mark. Hair morphology was studied in 8 patients with untreated acute alopecia areata and 3 normal adults without hair loss. Light microscopy, transmission and scanning electron microscopy revealed distinct structural differences in the distal end of hairs compared with the remainder of their length and with normal hair shafts. Transverse sections of hairs just below the frayed brush-like tip often displayed asymmetrical cortex disintegration. One side was compact and homogeneous while the other was deeply fissured and/or broken up into discrete heterogeneous-staining fragments of cortical, stratum corneum and cuticular components in addition to apparently degenerate cortex. Many exclamation mark hair tips lacked cuticle and had irregular profiles. Melanin was found in cortical and medullary fragments at the tip, although it was absent in the more degenerate forms of cortex. More proximal sections of these pathognomic telogen hairs revealed nearly normal hair shaft ultrastructure.

- Tomita K, Oda N, Ohbayashi M, Kamei H, Miyaki T, Oki T.
A new screening method for melanin biosynthesis inhibitors using Streptomyces bikiniensis. J Antibiot (Tokyo) 43:1601-1605, 1990.
Abstract : A novel screening method for melanin biosynthesis inhibitors using Streptomyces bikiniensis NRRL B-1049 as the indicator organism has been developed. Several known compounds, including tyrosinase inhibitors, were found to inhibit melanin production of S. bikiniensis on agar plates. This screening method was applied to fermentation broths of actinomycetes and several cultures with melanin biosynthesis inhibitory activity were found.

- Zillikens D, Mehringer A, Lechner W, Burg G.
Hypo- and hyperpigmented areas in incontinentia pigmenti. Light and electron microscopic studies. Am J Dermatopathol 13:57-62, 1991.
Abstract : Incontinentia pigmenti is a rare genodermatosis typically involving three stages: vesiculae, verrucous lesions, and hyperpigmentation. We clinically and pathologically documented a case from shortly after birth until the age of 17 years. Although the first two stages took a regular course, the third stage of the disease was characterized by hypopigmented streaks on the legs in addition to axillary hyperpigmentation. Similar hypopigmented areas were found in the patient's mother. Because pathological investigations of hypopigmented areas have been extremely rare, we performed light and electron microscopic studies and compared these with our findings in hyperpigmented regions. Light microscopy showed the hypopigmented streaks with slight epidermal atrophy and a reduced number of melanocytes and skin appendages. However, the main finding was round eosinophilic bodies in the upper dermis. Electron

microscopic examination of these bodies demonstrated amorphous material that resembled colloid, suggesting degeneration of basal keratinocytes. Confirming previous reports, in hyperpigmented areas we found a reduction of pigment in those parts of the basal layer overlying melanophages located in the upper dermis.

- Zuasti A, Ferrer C, Aroca P, Solano F.
Distribution of extracutaneous melanin pigment in Sparus auratus, Mugil cephalus, and Dicertranchus labrax (Pisces, Teleostei). Pigment Cell Res 3:126-131, 1990.
Abstract : The morphological and biochemical characteristics of pigment accumulations found in the kidney, liver, spleen, and mesentery of three different species of teleost fishes have been studied. There are significant differences in number, distribution, and morphology of pigment accumulations in different organs of the three species. Biochemical studies have shown the existence of tyrosinase activity in the mesentery of Mugil cephalus and in the kidney and mesentery of Sparus auratus. No tyrosinase activity was found in any internal organs of Dicertranchus labrax. That activity was assayed using three methods: tyrosine hidroxylation, dopa oxidation, and melanin formation. The morphological and biochemical observations are in agreement. In those organs in which we have demonstrated melanin synthetic activity, the pigment cells are morphologically and like melanophores, while in the organs that show no melanin synthetic activity, the pigment cells resemble macrophages.

3. MSH, MCH, other hormones, differentiation

- Baker BI, Kinsman RG, Moss CA, White PD, Paul PK, Brown DW, Campbell MM, Osguthorpe DJ.
Structure-activity studies with fragments and analogous of salmonid melanin-concentrating hormone. Peptides 11:1103-1108, 1990.
Abstract : A number of cyclic and linear fragments and analogues of MCH were synthesized and their biological potencies tested using the isolated carp scale melanophore assay. In this system the cyclic portion MCH(5-14) exhibited only 0.1% bioactivity, which was markedly enhanced by the addition of the exocyclic sequences MCH(15-17) and MCH(1-4). The exocyclic sequence itself, MCH(1-4,15-17), had minimal activity, however. Substitution of Tyr11 with phenylalanine reduced the potency of the ring structure MCH(5-14) by about 4-fold. Substitution of Gly8 with D-alanine reduced the potency of MCH(5-14) 16-fold, while both substitutions together caused a still more marked reduction (200-fold) in bioactivity. Linearized fragments of MCH, extending from MCH(15-17) to [Cys(Acm)5,14]MCH(1-17), showed a progressive increase in potency. The linearized forms of MCH, MCH(5-17) and MCH(5-14), were approximately 100-fold or less potent than their cyclic forms. The significant increases in bioactivity produced by the addition of the C- and N-terminal exocyclic sequence even to these linearized forms further emphasizes the importance of these regions for interaction at the receptor site.

- Baker BI.
Melanin-concentrating hormone: a general vertebrate neuropeptide. Int Rev Cytol 126:1-47, 1991.

- Batten TF, Cambre ML, Moons L, Vandesande F.
Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, Poecilia latipinna. J Comp Neurol 302:893-919, 1990.
Abstract : The comparative distribution of peptidergic neural systems in the brain of the euryhaline, viviparous teleost Poecilia latipinna (green molly) was examined by immunohistochemistry. Topographically distinct, but often overlapping, systems of neurons and fibres displaying immunoreactivity (ir) related to a range of neuropeptides were found in most brain areas. Neurosecretory and hypophysiotrophic hormones were localized to specific groups of neurons mostly within the preoptic and tuberal hypothalamus, giving fibre projections to the neurohypophysis, ventral telencephalon, thalamus, and brain stem. Separate vasotocin (AVT)-ir and isotocin (IST)-ir cells were located in the nucleus preopticus (nPO), but many AVT-ir nPO neurons also displayed growth hormone-releasing factor (GRF)-like-ir, and in some animals corticotrophin-releasing factor (CRF)-like-ir. The main group of CRF-ir neurons was located in the nucleus recessus anterioris, where coexistence with galanin (GAL) was observed in some cells. Enkephalin (ENK)-like-ir was occasionally present in a few IST-ir cells of the nPO and was also found in small neurons in the posterior tuberal hypothalamus and in a cluster of large cells in the dorsal midbrain tegmentum. Thyrotrophin-

releasing hormone (TRH)-ir cells were found near the rostromedial tip of the nucleus recessus lateralis. Gonadotrophin-releasing hormone (GnRH)-ir cells were present in the nucleus olfactoretinalis, ventral telencephalon, preoptic area, and dorsal midbrain tegmentum. Molluscan cardioexcitatory peptide (FMRF-amide)-ir was colocalized with GnRH-ir in the ganglion cells and central projections of the nervus terminalis. Melanin-concentrating hormone (MCH)-ir neurons were restricted to the tuberal hypothalamus, mostly within the nucleus lateralis tuberis pars lateralis, and somatostatin (SRIF)-ir neurons were numerous throughout the periventricular areas of the diencephalon. A further group of SRIF-ir neurons extending from the ventral telencephalon into the dorsal telencephalon pars centralis also contained neuropeptide Y (NPY)-, peptide YY (PYY)-, and NPY flanking peptide (PSW)-like-ir. These immunoreactivities were, however, also observed in non-SRIF-ir cells and fibres, particularly in the mesencephalon. Calcitonin gene-related peptide (CGRP)-like-ir had a characteristic distribution in cells grouped in the isthmal region and fibre tracts running forward into the hypothalamus, most strikingly into the inferior lobes. Antisera to cholecystokinin (CCK) and neurokinin A (NK) or substance P (SP) stained very extensive, separate systems throughout the brain, with cells most consistently seen in the ventral telencephalon and periventricular hypothalamus. Broadly similar, but much more restricted, distributions of cells and fibres were seen with antisera to neurotensin (NT) and vasoactive intestinal peptide (VIP).

- Bennett DC.
Color genes, oncogenes and melanocyte differentiation. J Cell Sci 98:135-139, 1991.
Abstract : A review and commentary with 40 refs. Pigmentation has long been a favorite and easily used marker in the genetics of diverse organisms. Mammalian integumental pigments are melanins, synthesized by melanocytes in the epidermis and hair bulbs. Our understanding of mammalian pigmentation genes has been advanced significantly in the last few years, partly by the advent of methods for the culture and immortalization of melanocytes. In cultured melanocytes, homozygous recessive germline mutations can be visibly expressed, complemented or even revert spontaneously. In the mouse there are >130 mutations that affect coat color, mapping to >50 loci. For the sake of brevity, most of this commentary will be concerned with the recent mol. characterization of mutations at just four of these loci, two of which encode developmentally controlled, melanocyte-specific products while the other 2 play a part in melanocyte development. The corresponding cancer, melanoma, makes an excellent system for the study of cell differentiation and its relationship to malignancy, partly again because of the ready visibility of melanin. The commentary will conclude with discussion of some relationships between malignancy and cell differentiation in melanocytes transformed by oncogenes or other agents.

- Birchall N, Orlow SJ, Kupper T, Pawelek J.
Interactions between ultraviolet light and interleukin-1 on MSH binding in both mouse melanoma and human squamous carcinoma cells. Biochem Biophys Res Commun 175:839-845, 1991.
Abstract : Interactions between beta-melanotropin (MSH), interleukin 1-a (IL-1), and ultraviolet light (UV) were examined in Cloudman S91 mouse melanoma and RHEK human squamous carcinoma cell lines. The following points were established: 1) both cell lines produced IL-1 and their production was stimulated by exposure of the cells to UV; 2) both cell lines possessed high affinity binding sites for MSH, and their ability to bind MSH was modulated by IL-1; 3) IL-1 exhibited both stimulatory and inhibitory effects on MSH binding to Cloudman cells; and 4) the stimulatory effect of IL-1 on MSH binding to melanoma cells was reflected in enhanced cellular responsiveness to MSH regarding tyrosinase activity (E.C. 1.14.18.1) and melanin content. The findings raise the possibility that interactions between keratinocytes and melanocytes may be regulated by IL-1 and MSH, and suggest a possible mechanism for stimulation of cutaneous melanogenesis by solar radiation: enhancement of MSH receptor activity by induction of IL-1.

- Bugnon C, Bahjaoui M, Fellmann D.
A simple method for coupling in situ hybridization and immunocytochemistry: application to the study of peptidergic neurons. J Histochem Cytochem 39:859-862, 1991.
Abstract : We have devised a simple procedure for immunostaining of sections that have previously undergone autoradiographic visualization of mRNAs by in situ hybridization. Classical hybridocytochemistry techniques were performed first on cryostat sections of formaldehyde-fixed tissue. Standard methods were used for slide coating by emulsion dipping and for revelation, fixation, and coverslipping steps. The key to this method is the emulsion removal, or permeabilization, by a short trypsin incubation (0.2% for 20-30 sec)

which facilitates the good access of antibodies used in a subsequent immunocytochemical technique to section epitopes. Usual immunofluorescence and immunoperoxidase procedures were successfully performed after this treatment. The immunoreactivity of several neuropeptides was well preserved after this procedure. In addition to its usefulness in our studies, this general method should be applicable to many other situations in which autoradiographic and immunocytochemical detections must be coupled.

- Cakir S, Koksal F, Tapramaz R, Cakir O.
Electron spin resonance of free radicals in some legumes, cereals and their aqueous solutions under photolysis. Radiat Phys Chem 38:17-21, 1991.
Abstract : In this study, free radicals producerd by u.v. photolysis in some legumes and cereals were investigated by ESR (ESR). The ESR spectra of small grains and powd. legume and cereal samples were investigated at room temp. before and during photolysis. Before photolysis, barley, wheat, rye, oat, and lentil samples exhibited the well known spectra of Mn2+ in addn. to a central signal of g = 2.0045 which is attributed to the melanin radical. The melanin signal was obsd. also more clearly, in the samples of bean, chickpea, and maize shells before photolysis. The melanin signal was obsd. during photolysis in wheat, barley, oat, maize, rice, bean, lentil, and chickpea samples at room temp. Furthermore, it aq. solns., all the cereals and legumes investigated in this study gave HCO and CO2- radicals at 123K under photolysis. HCO and CO2- radicals originated from the glucose mols. in the carbohydrate chains of these samples.

- Chakraborty AK, Orlow SJ, Bolognia JL, Pawelek JM.
Structural/functional relationships between internal and external MSH receptors: modulation of expression in Cloudman melanoma cells by UVB radiation. J Cell Physiol 147:1-6, 1991.
Abstract : Expression of internal receptors for MSH is an important criterion for responsiveness to MSH by Cloudman melanoma cells (Orlow et al: J. Cell. Physiol., 142:129-136, 1990). Here, we show that internal and external receptors for MSH are of identical molecular weights (50-53 kDa) and share common antigenic determinants, indicating a structural relationship between the 2 populations of molecules. The internal receptors co-purified with a sub-cellular fraction highly enriched for small vesicles, many of which were coated. Ultraviolet B light (UVB) acted synergistically with MSH to increase tyrosinase activity and melanin content of cultured Cloudman melanoma cells, consistent with previous findings in the skin of mice and guinea pigs (Bolognia et al: J. Invest. Derm., 92:651-656, 1989). Preceding the rise in tyrosinase activity in cultured cells, UVB elicited a decrease in internal MSH binding sites and a concomitant increase in external sites. The time frame for the UVB effects on MSH receptors and melanogenesis, 48 hours, was similar to that for a response to solar radiation in humans. Together, the results indicate a key role for MSH receptors in the induction of melanogenesis by UVB and suggest a potential mechanism of action for UVB: redistribution of MSH receptors with a resultant increase in cellular responsiveness to MSH.

- Eberle AN, Verin VJ, Solca F, Siegrist W, Kueenlin C, Bagutti C, Stutz S, Girard J.
Biologically active monoiodinated .alpha.-MSH derivatives for receptor binding studies using human melanoma cells. J Recept Res 11:311-322, 1991.
Abstract : Three different monoiodinated radioligands of .alpha.-MSH were compared in a binding assay with human D10 melanoma cells: [Tyr(125I)2]-.alpha.-MSH, [Tyr(125I)2,Nle4]-.alpha.-MSH, and [Tyr(125I)2,Nle4,D-Phe7]-.alpha.-MSH. They were prepd. either by the classical chloramine T method or by the Enzymobead method. A simple and rapid purifn. scheme was developed consisting of a primary sepn. on reversed-phase C18 silica cartridges immediately after the iodination, followed by HPLC purifn. before each binding expt. Biol. testing of the three radioligands showed that they all retained high melanotropic activity in the B16 melanin assay and the Anolis melanophore assay. However, in human D10 melanoma cells, [Tyr(125I)2,Nle4]-.alpha.-MSH led to a high degree of non-specific binding to the cells which could not be displaced by excess .alpha.-MSH and only partially by [Nle4]-.alpha.-MSH. The [Tyr(125I)2,Nle4,D-Phe7]-.alpha.-MSH tracer gave similar results but with a much lower proportion of non-specific binding. On the other hand, [Tyr(125I)2]-.alpha.-MSH proved to be an excellent radioligand whose non-specific binding to the D10 cells was not higher than 20% of the total binding.

- Fischer WH, Vaughan J, Nahon JL, Presse F, Hoeger C, Rivier JE, Vale W.
Purification and sequence analysis of rat melanin concentrating hormone. Pept Chem, Struct Biol, Proc Am Pept Symp, 11th, Meeting Date 1989, 464-7. Edited by: Rivier, Jean E.; Marshall, Garland R. ESCOM Sci.

Pub.: Leiden, Neth., 1990.
Abstract : A symposium report on the purifn. and sequence anal. of rat melanin concg. hormone.

- Granholm NH, Van Amerongen AW.
Effects of exogenous MSH on the transformation from phaeo- to eumelanogenesis within C57BL/6J-Ay/a hairbulb melanocytes. J Invest Dermatol 96:78-84, 1991.
Abstract : The extent to which alpha melanocyte-stimulating hormone (MSH) is a true in vivo regulator of melanogenesis in mice is unknown. The objective of this study was to determine if MSH-induced eumelanogenesis in hairbulb melanocytes of yellow (Ay/a) mice mimics the natural program of eumelanogenesis occurring in genetically black (a/a) hairbulb melanocytes. We conducted quantitative transmission electron microscopy on melanosome differentiation within MSH-treated regenerating 9-d hairbulbs of Ay/a and a/a mice. Results of exogenous alpha-MSH injections (5 d at 0.15 mM MSH) showed that the striking visual darkening of hair was accompanied by an incomplete transformation of phaeo- to eumelanogenesis. Ontogenetic data on developmental stages I-IV of 3678 melanosomes based on geometric considerations (length, width, shape, and area) showed that MSH did not induce a complete transformation from spherical phaeomelanosomes to elliptical eumelanosomes. Also, observations on the number of vesiculoglobular bodies and matrix organization reveled that MSH-treated Ay/a melanosomes retained distinct features of phaeomelanogenesis even after 5 d of MSH treatment. Thus, MSH induced a partial but incomplete pattern of eumelanogenesis in regenerating hairbulb melanocytes of Ay/a mice. The continued investigation of the dynamics of melanin synthesis in MSH-induced Ay/a mice melanocytes possessing "mosaic" melanosomes could be productive in understanding fundamental relationships between tyrosinase activity, matrix function, matrix structure, and regulation of melanin (phaeo- and/or eumelanin) synthesis.

- Green JA, Baker BI.
The influence of repeated stress on the release of melanin-concentrating hormone in the rainbow trout. J Endocrinol 128:261-266, 1991.
Abstract : Melanin-concentrating hormone (MCH) is a neurohypophysial peptide that induces pigmentary pallor in teleosts and which is released when the fish are placed on a white background. An additional effect of the peptide is the depression of ACTH and hence cortisol secretion during moderate stress. The present work on rainbow trout shows that plasma MCH concentrations, while unaffected by a single stress, are raised by repeated stress (1 ml saline injected i.p. without anaesthesia) and remain high for several hours thereafter. The response to stress is observed only in white-adapted fish and not in fish kept in black-coloured tanks, when MCH release is normally low. Plasma concentrations of MCH vary diurnally but stress induces an equivalent incremental rise in plasma MCH, whether administered in the middle or towards the end of the photophase. The stress-induced rise in MCH concentrations is prevented by treatment with dexamethasone. The results support the suggestion that the modulatory effect of MCH on the hypothalamopituitary-interrenal axis of fish might be enhanced under conditions of stress.

- Green JA, Baker BI, Kawauchi H.
The effect of rearing rainbow trout on black or white backgrounds on their secretion of melanin-concentrating hormone and their sensitivity to stress. J Endocrinol 128:267-274, 1991.
Abstract : Rainbow trout were reared in black or off-white coloured tanks for up to 18 months of age to achieve maximum differences in the synthesis of the neuropeptide, melanin-concentrating hormone (MCH). White-reared fish had greatly increased MCH concentrations in their pituitary glands, in their MCH perikarya and in the presumptive neuromodulatory fibres of the dorsal hypothalamus/thalamus when compared with black-reared and commercially reared trout. Following transfer to brighter white tanks, white-reared fish showed a significant increase in plasma MCH concentration and a reduction of MCH in the pituitary and MCH perikarya. The additional challenge of repeated stress further increased plasma MCH concentration in these fish and also reduced MCH in the dorsal hypothalamus/thalamus. In black-reared fish transferred to white tanks, plasma MCH concentrations were significantly raised after transfer, although they were lower after 11 days than in white-reared counterparts. Transfer from black to white background caused a fall in the MCH concentration in all regions--pituitary gland, perikarya and dorsal hypothalamus/thalamus; if transfer was accompanied by repeated stress, the hormone in the pituitary gland and MCH perikarya became so depleted that plasma MCH concentrations declined. Within each experimental situation (control, background transfer and transfer with stress) there was in inverse correlation between plasma MCH

concentrations of black- and white-reared fish and the cortisol concentration. MCH had no direct effect on the secretion of cortisol by interrenal tissue but incubated hypothalami, in which endogenous MCH had been immunoabsorbed, provided evidence that MCH can depress the release of corticotrophin-releasing bioactivity.

- Matsunaga TO, Gehrig CA, Hruby VJ.
1H-NMR assignments and conformational studies of melanin concentrating hormone in water using two-dimensional NMR. Biopolymers 30:1291-1295, 1990.
Abstract : Total correlation spectroscopy, phase sensitive NOE, and double quantum filtered phase-sensitive correlated spectroscopy were used to assign backbone and side chain protons of salmon melanin concg. hormone (I) in 90% H2O/10% O2O. NMR spectroscopy identified a .beta.-turn region in the I mol as well as a transannular effect of the Tyr11 side-chain moiety. The prominent i - i + 2 CH2-NH NOE of medium proportion and the subsequent NH-NH and CH2-CH2 interactions between residues 7 and 10 indicate that a type I .beta.-turn exists in this region; this is supported by distance geom. calcns.

- Matsunaga TO, Gehrig CA, Hruby VJ.
Proton NMR assignments and conformational studies of melanin-concentrating hormone in water using NOE constraints and molecular modeling algorithms. Pept Chem, Struct Biol, Proc Am Pept Symp, 11th, Meeting Date 1989, 600-2. Edited by: Rivier, Jean E.; Marshall, Garland R. ESCOM Sci. Pub.: Leiden, Neth, 1990.
Abstract : A symposium report on the conformational anal. of salmon melanin-concg. hormone (I) in H2O using 2D NMR and mol. modeling methods. This study was used to det. the conformation structure requirement for I at its receptor.

- Moss CA.
Structure-conformation-activity studies of the melanin concentrating hormone (MCM). Avail. Univ. Microfilms Int., Abstr Int B 51:3827, 1991.

- Seechurn P, Thody AJ.
The effect of ultraviolet radiation and melanocyte-stimulating hormone on tyrosinase activity in epidermal melanocytes of the mouse. J Dermatol Sci 1:283-288, 1990.
Abstract : The role of MSH as a mediator of the melanogenic response to UV radiation was examd. in C57 BL/6 mice. While exposure to UV (250-300 nm) for 7, 14, and 27 days increased tyrosinase activity in epidermal melanocytes of the ear, MSH had no effect and failed to alter the response to UV. Plasma .alpha.-MSH concns. were unchanged following UV. Theophylline, a phosphodiesterase inhibitor, also had no effect on epidermal tyrosinase activity in nonirradiated and UV irradiated mice. Prostaglandin E2 and arachidonic acid were also ineffective in nonirradiated and UV irradiated mice and indomethacin, an inhibitor of prostaglandin synthesis, failed to increase epidermal tyrosinase activity after UV. On the other hand, 12-O-tetradecanoyl phorbol-13-acetate, an activator of protein kinase C, increased epidermal tyrosinase activity in nonirradiated mice and also enhanced the effect of UV.

- Svensson SP, Norberg T, Andersson RG, Grundstrom N, Karlsson JO.
MCH-induced pigment aggregation in teleost melanophores is associated with a cAMP reduction. Life Sci 48:2043-2046, 1991.
Abstract : It has previously been shown that alpha 2-adrenoceptors are involved in noradrenaline-induced pigment aggregation within fish melanophores. In the present investigation, melanin concentrating hormone (MCH) elicited pigment aggregation (EC50 approximately 1 x 10(-7) M) that was associated with a significant reduction in the cAMP content; 1 x 10(-7) M MCH reduced the cAMP content from a basal level of 50.4 +/- 2.8 pmol/mg protein to 36.9 +/- 3.8 pmol/mg protein. Like the alpha 2-adrenoceptor-induced pigment aggregation, the MCH response was effectively blocked by the adenylate cyclase stimulator forskolin. These findings suggest that attenuation of cAMP may serve as an intracellular signal transduction mechanism for both MCH and noradrenaline.

4. Photobiology and photochemistry

- Bernstein EF, Thomas GF, Smith PD, Mitchell JB, Glatstein E, Kantor GR, Spielvogel RL, Maiese SC, Russo

A.
Response of black and white guinea pig skin to photodynamic treatment using 514-nm light and dihematoporphyrin ether. Arch Dermatol 126:1303-1307, 1990.
Abstract : Differences in skin pigmentation may affect light penetration during photodynamic therapy. This study evaluated the effect of skin pigmentation on the dermatotoxic reaction to photodynamic therapy utilizing the photosensitizer DHE. Black and white guinea pigs were given 10 mg/kg of DHEA, depilated, and treated 48 h after injection with 30 mW/cm2 of 514-nm light. Eschar formation was obsd. on white skin at an av. light dose of 26 J/cm2, whereas black skin showed similar changes at 58 J/cm2. Microscopically, superficial necrosis corresponded to the gross changes noted. The results agree with data describing the difficulty of treating pigmented lesions such as malignant melanoma with photodynamic therapy. This further suggests that higher light doses may be required to treat superficial lesions and produce skin photosensitivity in dark-skinned individuals.

- Hill HZ.
Melanins in the photobiology of skin cancer and the radiobiology of melanomas. Cancer Biol Biosynth, pp. 31-53. Edited by: Wilson, Samuel H. CRC: Boca Raton, Fla., 1991.

- Kollias N, Sayre RM, Zeise L, Chedekel MR.
Photoprotection by melanin. J Photochem Photobiol, B 9:135-60, 1991.
Abstract : A review with 105 refs. on the current state of information on melanin and epidermal melanin pigmentation (EMP) as photoprotective agents. The chem. and biochem. of melanin (the particle) and its interaction, in its various forms, with UV radiation are considered. Methods of attenuation of UV radiation are discussed in terms of structure and chem. constituents. Photoprotection by constitutive and facultative pigmentation is reviewed with min. erythema dose (MED) as the end point. The issue of acclimatization to UV radiation is discussed in terms of UVB phototherapy for psoriasis. Finally, skin cancer is considered as an end point and the redn. of its incidence with pigment level is discussed. It is concluded that while EMP provides protection, its extent depends on the end point chosen for evaluation. MED is a convenient photobiol. end point but is rather insensitive, whereas skin cancer is sensitive but impractical for lab. studies. Our current state of knowledge of melanin lacks information on its absorption and scattering coeffs. and its refractive index. Methods for the quant. measurement of EMP are also urgently required.

- Pawlowski A, Pawlowski MD, Lea PJ.
Effects of UV radiation on the ultrastructure of human common pigmented naevi and lentigines. Acta Derm Venereol (Stockh) 71:113-117, 1991.
Abstract : In order to investigate how sunlight may affect naevi and lentigines, their melanocytes and the basement membrane, three irradiation protocols were applied directly to ten naevi and five lentigines on 2 subjects. Neither volunteer had sufficient naevi and lentigines to be able to use the three irradiation protocols on each of the subjects. Skin biopsies were fixed in glutaraldehyde followed by osmium tetroxide, thin-sectioned and examined in a Hitachi H-7000 transmission electron microscope. Following 14 consecutive single exposures of 3 MED of UVB or single exposures followed by 25 J/cm2 of UVA, 350 J/cm2 UVA with either 2040 or 2280 mJ/cm2 UVB, the basement membrane maintained its continuity. Melanocytes were not observed on the dermal side of the epidermal-dermal junction. UVA irradiation stimulated reinforcement of the basement membrane zone by collagen fibers. Centrioles found in melanocytes following irradiation suggest that these melanocytes maybe undergoing mitosis. Dermal fibroblasts were found to contain comparatively large quantities of melanin pigment. The pigment contained in these fibroblasts may in fact constitute an additional barrier against UV irradiation.

5. Neuromelanins

- Gibb WR, Scott T Lees AJ.
Neuronal inclusions of Parkinson's disease. Mov Disord 6:2-11, 1991.
Abstract : Two distinct neuronal inclusions occur in Parkinson's disease. The Lewy body is the diagnostic hallmark and is recognized by its eosinophilic body and unstained halo. It can be found in specific regions of the nervous system, where its frequency, size, shape, and structure differ. Large neurons of the dorsal vagal

nucleus and sympathetic ganglia often contain particularly large quantities of Lewy-body-like matter. It consists of filament in the outer part and electron dense material in the core, the outer part staining with silver and with antibodies to neurofilament and tubulin. The pale body is restricted to the substantia nigra and locus ceruleus. It does not react with conventional stains, silver, or neurofilament antibodies, and has a homogeneous structure with a granular and vesicular surface texture. It contains sparse granular matter, vacuoles, and filaments, surrounded by melanin. The Lewy body and pale body may be juxtaposed or contiguous in some cells, but their distinct appearances and structures indicate that they are separate inclusions.

- Hirsch EC, Brandel JP, Galle P, Javoy-Agid F, Agid Y.
Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis. J Neurochem 56:446-451, 1991.
Abstract : The levels of different elements were studied by x-ray microanalysis in the substantia nigra and the central gray substance of patients with Parkinson's disease, progressive supranuclear palsy, and matched controls. In control brains, only iron, potassium, silicum, sodium, sulfur, and zinc were within the limit of detection of the technique. The abundance of each element was different, but their respective concentrations in the two brain regions were similar, except for sulfur levels which were higher on neuromelanin aggregates in the substantia nigra than in nigral regions lacking neuromelanin, and in the central gray substance. In Parkinson's disease, but not in progressive supranuclear palsy, nigral iron levels increased in regions devoid of neuromelanin and decreased on neuromelanin aggregates, but were unchanged in the central gray substance, when compared to control values. Concentrations of the other elements in the central gray substance and substantia nigra were not different from controls in brains from patients with Parkinson's disease and progressive supranuclear palsy. Analysis of Lewy bodies in the parkinsonian substantia nigra revealed high levels of iron and the presence of aluminum. Metal abundance was not affected in progressive supranuclear palsy, in spite of the nigral cell death. This suggests that the increased iron levels and the detection of aluminum observed in Parkinson's disease are not solely the consequence of the neuronal degeneration.

- Iwabuchi K, Yagishita S, Amano N, Yokoi S, Honda H, Tanabe T, Kinoshita J, Kosaka K.
An autopsy case of complicated form of spastic paraplegia with amyotrophy, mental deficiency, sensory impairment, and parkinsonism. No To Shinkei 42:1075-1083, 1990.
Abstract : An autopsied case of complicated form of spastic paraplegia with many unusual clinical and pathological features is reported. Present case: a 31-year-old male. His parents are first cousins. Pregnancy and delivery had been unremarkable. Though he was mentally retarded, his physical development was normal. He was considered normal until age 10. He suffered from progressive disturbance in gait at the age of 11. He could not walk without assistance at the age of 22. Neurological examination revealed the following findings. He was obese and mentally deteriorated. Spastic paraplegia with increased tendon reflexes and pathological reflexes was prominent. Though slight sensory disturbance was present in the lower extremities, neither involuntary movement nor cerebellar ataxia was observed. In the age of late 20's, dementia, general muscular atrophy, and Parkinsonism developed. At the age of 30, he could not move by himself. He was apathic and indifferent, and showed forced laughing. Muscle tonus was flaccid because of general muscular atrophy and peripheral neuropathy. He died of acute gastric enlargement. Neuropathological findings were characterized by mal-development of the central nervous system (CNS) and the multisystem degeneration. There existed cerebral white matter hypoplasia with hypogenesis of the corpus callosum and ectopia of neurons of the cerebral and cerebellar cortex. Hypoplasia of melanin pigment was also observed in the remaining neurons of the substantia nigra and the locus ceruleus. Many neurons in the CNS included lipofuscin granules of variable shapes. Some of them showed clusters of several block-like inclusions which were green with luxol fast blue and cresyl violet stain.

- Jellinger K, Paulus W, Grundke-Iqbal I, Riederer P, Youdim MB.
Brain iron and ferritin in Parkinson's and Alzheimer's diseases. J Neural Transm Park Dis Dement Sect 2:327-340, 1990.
Abstract : Semiquantitative histological evaluation of brain iron and ferritin in Parkinson's (PD) and Alzheimer's disease (DAT) have been performed in paraffin sections of brain regions which included frontal cortex, hippocampus, basal ganglia and brain stem. The results indicate a significant selective increase of

Fe3+ and ferritin in substantia nigra zona compacta but not in zona reticulata of Parkinsonian brains, confirming the biochemical estimation of iron. No such changes were observed in the same regions of DAT brains. The increase of iron is evident in astrocytes, macrophages, reactive microglia and non-pigmented neurons, and in damaged areas devoid of pigmented neurons. In substantia nigra of PD and PD/DAT, strong ferritin reactivity was also associated with proliferated microglia. A faint iron staining was seen occasionally in peripheral halo of Lewy bodies. By contrast, in DAT and PD/DAT, strong ferritin immunoreactivity was observed in and around senile plaques and neurofibrillary tangles. The interrelationship between selective increase of iron and ferritin in PD requires further investigation, because both changes could participate in the induction of oxidative stress and neuronal death, due to their ability to promote formation of oxygen radicals.

- Marinkovic S.
Morphological characteristics of the A10 catecholaminergic group of neurons in the human midbrain. Anat Anz 171:115-124, 1990.
Abstract : The authors examined ten serially sectioned human midbrains stained with luxol fast blue and/or cresyl violet. They found the neuromelanin-containing neurons in the central (CL) and rostral (RL) linear nuclei, the interfascicular (IF), the paranigral (PN), and the parabrachial pigmented (PB) nuclei, as well as in the medial longitudinal fasciculus and the dorsal nucleus of the raphe. The CL nucleus measured 4.7 mm x 1.9 mm, the RL 2.9 mm x 0.6 mm, the IF 2.8 mm x 0.6 mm, the PN 1.3 mm x 0.8 mm, and the PB 4.4 mm x 0.7 mm. The number of pigmented neurons per section was 9.4 in the CL, 13.5 in the RL, 51.7 in the IF, 41.8 in the PN, and 33.1 in the PB nucleus. The pigmented neurons, which were fusiform, oval or multipolar, ranged from 9.3 microns x 9.0 microns to 62.0 microns x 25.0 microns in size. Clustering of the cells was most prominent in the IF and PN nuclei, as well as in the lateral parts of the PB and RL nuclei. The authors concluded that: 1. the CL and PB were the largest nuclei; 2. the greatest cellular density was in the IF and PN nuclei; 3. the largest pigmented neurons were present in the RL and PB nuclei, and 4. the CL and RL nuclei were more complex than the other nuclei of the A10 catecholaminergic group.

- Metodiewa D, Dunford HB.
Involvement of lactoperoxidase in the peroxidative degradation of serotonin: a potential pathway for indoleaminergic melanin formation. Biochem Int 23:183-191, 1991.
Abstract : The peroxidase-catalyzed degrdn. of 5-hydroxytryptamine (serotonin) was studied using rapid scan of conventional spectrophotometry for detection of one-electron conversions of lactoperoxidase I, II, and III. The spectral changes of serotonin during oxidn. and spectral and bleaching properties of reaction products were examd. The results of the investigation clearly indicate the ability of serotonin to function as an electron donor substrate for animal peroxidases.

- Mizutani Y, Yokochi M, Oyanagi S.
Juvenile parkinsonism: a case with first clinical manifestation at the age of six years and with neuropathological findings suggesting a new pathogenesis. Clin Neuropathol 10:91-97, 1991.
Abstract : A clinico-pathological study of a 39-year-old female with Juvenile parkinsonism was carried out. Although the clinical manifestations were consistent with parkinsonism, the pathological findings were significantly different. Pathological examination showed the lesion to be localized to the substantia nigra, the number of neurons to be abnormally low, the proportion of melanin-containing cells to be reduced, and a large number of immature cells to be present. No neuronal degeneration associated with gliosis or release of melanin granules, such as seen in Parkinson's disease, was observed. Neuropathological studies, including cytometry and comparison against normal controls in the distribution of melanin granules, suggested hypoplasia and poor neuronal maturation of the substantia nigra. Since neuromelanin is thought to be the result of normal dopamine metabolism, reduction of melanin-containing cells in this case suggests inadequate or abnormal dopamine metabolism. Thus, the clinical manifestation of parkinsonism in this case seems to be related to the small number of melanin-containing cells which implies the dopamine deficiency state.

- Moll G, Moll R, Riederer P, Gsell W, Heinsen H, Denney RM.
Immunofluorescence cytochemistry on thin frozen sections of human substantia nigra for staining of monoamine oxidase A and monoamine oxidase B: a pilot study. J Neural Transm Suppl 32:67-77, 1990.

- Nishinaka T, Kuroda S, Hayashi Y, Fujisawa Y.
Motor neuron disease with Parkinson's disease--case report. Rinsho Shinkeigaku 30:1252-1255, 1990.
Abstract : The patient was a 64-year-old woman who showed muscle weakness and tremor of upper extremities and gait disturbance at the age of 62 years. The symptoms progressed and she was admitted to our hospital. Neurological examination revealed muscle weakness, muscle atrophy and fasciculation bilaterally in the upper extremities. The deep tendon reflexes were reduced in the upper extremities and increased in the lower extremities, but Babinski's sign was not present. There was mild hand tremor at rest (right greater than left). Muscle rigidity was also evident. Her gait was small-stepped and her trunk was bent forward. She showed hypomimia, but no dementia was detected. She died of respiratory failure 7 months after admission. The duration of the illness was about 2 years. At autopsy, macroscopic examination showed depigmentation of the substantia nigra and locus ceruleus, and atrophy of the anterior roots of the spinal cord. Microscopic examination revealed a few senile plaques in the temporal cortex. In the substantia nigra, the number of melanin-containing cells was decreased in its central parts. A few Lewy bodies were found in some of the remaining neurons, and melanin pigment migrated into the parenchyma. In the locus ceruleus and dorsal motor nucleus of vagus, abundant Lewy bodies and mild astrocytosis were seen. A few Lewy bodies were also seen in the nucleus raphe, nucleus basalis of Meynert and hypothalamic nuclei. Severe neuronal loss of the anterior horn cells was observed in the cervical segment, and to a lesser degree, in the lumbo-sacral segments.

- van Domburg PH.
The human substantia nigra and ventral tegmental area. A neuroanatomical study with notes on aging and aging diseases. Adv Anat Embryol Cell Biol 121:1-132, 1991.
Abstract : The present study comprises a cytoarchitectonic analysis of the human substantia nigra (SN) and ventral tegmental area (VTA); a discussion of their chemoarchitecture and fiber connections (mainly based on tract-tracing studies in primates) preceded by an overview of the wealth of tract-tracing data in rodents; a discussion of the involvement of the SN/VTA complex in Parkinson's disease (PD) and related disorders and in Alzheimer's disease (AD), including some quantitative data; and finally, some functional and pathophysiological considerations, relating nigral organization to pathophysiology and hypotheses on the etiology and distribution of AD and PD. DAergic cell populations in the mesencephalon (SN pars compacta, VTA, and the retrorubral area A8) which give rise to well-developed, DAergic, mesotelencephalic pathways, including a distinct mesostriatal system, and a substance P-immunoreactive striatotegmental system which projects to the SN pars reticulata and VTA appear to be common to reptiles, birds, and mammals (Sect. 3.1). The extensive literature on the organization of the SN/VTA complex in rats is summarized in Sect. 3.2. The mesotelencephalic projection is organized along inverted dorsal to ventral, medial to lateral, and rostral to caudal topographies. A dense DAergic innervation is characteristic of the entire striatal complex, including the caudate-putamen (the dorsal striatum), the nucleus accumbens, and the olfactory tubercle (the ventral striatum). This mesostriatal projection is compartmentally organized with distinct sets of DAergic neurons projecting to striosomes and extrasriosomal matrix, respectively, suggesting specialized channels directed at DAergic modulation of sensorimotor processing in the striatal matrix and limbic related mechanisms represented in the striosomal system. The VTA and medial part of the SN give rise to the DAergic mesolimbocortical system with extensive projections to limbic, allocortical, and neocortical structures. The striatonigral output pattern in rats is organized in such a way that the dorsal striatum mainly innervates the SN pars reticulata, whereas the ventral striatum projects predominantly to the VTA and medial part of the SN. Within the striatonigral projections in rats some interesting channels can be recognized, relating the sensorimotor cortex, via its corticostriatal projections, to that region of the SN giving rise to the nigrothalamic projection, and the visual cortex to the nigrotectal component of the SN pars reticulata.

6. Genetics

- Chintamaneni CD, Ramsay M, Colman MA, Fox MF, Pickard RT, Kwon BS.
Mapping the human CAS2 gene, the homologue of the mouse brown (b) locus, to human chromosome 9p22-pter. Biochem Biophys Res Commun 178:227-235, 1991.
Abstract : Melanin biosynthesis is a multistep process with the first step being the conversion of L-tyrosine to L-Dopa catalyzed by the enzyme tyrosinase. The enzymes which catalyze the other steps of melanogenesis

are not known. One murine pigmentation gene, the brown (b) locus, when mutated, leads to a brown or hypopigmented coat. The b-locus protein has been shown to display catalase activity. The human b-locus, therefore, is designated as CAS2. We used the mouse b-locus cDNA to isolate the human homologue, which in turn, was used to map the CAS2 locus to a human chromosome. The potential CAS2 protein codes for 527 amino acids containing a putative signal sequence and transmembrane domain. The CAS2 protein has primary and probably secondary structures similar to human tyrosinase. The CAS2 was mapped to human Chromosome 9 by somatic cell hybridization and, more specifically, to 9p22-pter by in situ hybridization. The assignment of CAS2 on the human Chromosome 9 extends this region of known homology on mouse Chromosome 4.

- Giebel LB, Tripathi RK, King RA, Spritz RA.
A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. A human homologue to the Siamese cat and the Himalayan mouse. J Clin Invest 87:1119-1122, 1991.
Abstract : Type I oculocutaneous albinism (OCA) is an autosomal recessive disorder in which deficient synthesis of melanin pigment results from abnormal activity of melanocyte tyrosinase. A novel type I OCA phenotype in which hypopigmentation is related to local body temperature is associated with a missense substitution in tyrosinase, codon 422 CGG (Arg)----CAG (Gln). This substitution results in a tyrosinase polypeptide that is temperature-sensitive. This form of type I OCA thus is homologous to the temperature-related forms of albinism seen in the Siamese cat and the Himalayan mouse.

- Jimenez M, Tsukamoto K, Hearing VJ.
Tyrosinases from two different loci are expressed by normal and by transformed melanocytes. J Biol Chem 266:1147-1156, 1991.
Abstract : Two pigmentation related genes have recently been cloned which map to the brown (b) and albino (c) loci of mice; these loci influence the quality and quantity, respectively, of melanin produced by melanocytes. Both these gene products are biochemically similar and have extensive amino acid sequence similarity to each other and to lower forms of tyrosinase (EC 1.14.18.1), a copper binding enzyme responsible for melanin production. In order to characterize the catalytic activities of these molecules, we have synthesized peptides and prepared antibodies to them which specifically recognize the gene products in question. By use of immune affinity purification protocols, we have isolated the proteins encoded by the brown and albino loci and have determined that both have the catalytic functions ascribed to tyrosinase, i.e. hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone. These are the critical reactions to melanogenesis since melanin pigment can be spontaneously produced from those products. The specific activity of the albino locus encoded product is considerably higher than that of the protein encoded by the brown locus, although the latter protein is present in higher quantity in melanocytes than is the protein encoded by the albino locus. These results are surprising since it was anticipated that tyrosinase was the product of single gene locus, and suggest that regulation of melanogenesis in mammals is controlled at the enzymatic level by several different gene products.

- Kluppel M, Beermann F, Ruppert S, Schmid E, Hummler E, Schutz G.
The mouse tyrosinase promoter is sufficient for expression in melanocytes and in the pigmented epithelium of the retina. Proc Natl Acad Sci USA 88:3777-3781, 1991.
Abstract : The mouse c locus encodes tyrosinase (monophenol monooxygenase; monophenol, L-dopa:oxygen oxidoreductase, EC 1.14.18.1), the key enzyme in melanin synthesis, which is expressed in the pigment epithelium of the retina and in melanocytes derived from the neural crest. To define regulatory regions of the gene that are important for cell type-specific expression, a deletion series of the tyrosinase 5' region was fused to a chloramphenicol acetyltransferase (CAT) reporter gene and electroporated into tyrosinase-expressing and -nonexpressing cell lines. We show that 270 base pairs 5' of the transcriptional start site is sufficient for CAT expression in a human and a mouse melanoma cell line. This 5' flanking fragment, when cloned in the context of a tyrosinase minigene construct and injected into fertilized eggs of an albino mouse strain, is sufficient for cell type-specific expression in mice. The transgenic mice were pigmented in both skin and eyes. In situ hybridization analysis shows that the 270-base-pair regulatory region contains elements sufficient for specific expression of the transgene both in the pigmented epithelial cells of the retina, which are derived from the optic cup, and in neural crest-derived melanocytes.

- Oetting WS, Mentink MM, Summers CG, Lewis RA, White JG, King RA.
Three different frameshift mutations of the tyrosinase gene in type IA oculocutaneous albinism. Am J Hum Genet 49:199-206, 1991.
Abstract : Mutations in the gene for the pigment-producing enzyme tyrosinase are responsible for type IA (tyrosinase-negative) oculocutaneous albinism (OCA). Most reported mutations have been single base substitutions. We now report three different frameshift mutations in three unrelated individuals with type IA OCA. The first individual has a single base deletion within a series of five guanidines, resulting in a premature stop codon in exon I on one allele and a missense mutation at codon 382 in exon III on the homologous allele. The second individual is a genetic compound of two separate frameshift mutations, including both the same exon I single base deletion found in the first individual and a deletion of a thymidine-guanidine pair, within the sequence GTGTG, forming a termination codon (TAG) in exon I on the homologous allele. The third individual has a single base insertion in exon I on one allele and a missense at codon 373 in exon III on the homologous allele. The two missense mutations occur within the copperBbinding region and may interfere with either copper binding to the enzyme or oxygen binding to the copper. These five different mutations disrupt tyrosinase function and are associated with a total lack of melanin biosynthesis.

- Takeuchi T.
Mammalian pigment cells. Differentiation and gene function. Iden 44:25-30, 1990.
Abstract : A review, with 3 refs., on the differentiation and genetics of mammalian pigment cells, esp. the melanin-synthesizing melanocytes, discussing the genesis, differentiation, and genes of melanocytes, pigment pattern formation, and the mol. biol. of pigment cells.

- Thompson RC, Watson SJ.
Nucleotide sequence and tissue-specific expression of the rat melanin concentrating hormone gene. DNA Cell Biol 9:637-645, 1990.
Abstract : Melanin concentrating hormone (MCH) is a key neuroendocrine peptide which is involved in the regulation of body color in teleost fish. Antigenically similar peptides exist in higher vertebrates including rodents and man. The precise function(s) of these peptides in these higher vertebrates has yet to be fully elucidated, although regulatory roles in stress-induced or corticotropin-releasing hormone-stimulated ACTH release and/or water balance have been proposed. The salmon, rat, and human MCH cDNA clones have been isolated and sequenced. We isolated and characterized the structure of the rat MCH gene. In addition to providing the complete nucleotide sequence of this gene, we demonstrate that there is a single copy of this gene in the rat genome. The structure of the rat MCH gene indicates that the MCH mRNA is encoded by three exons. Using primer extension and RNase protection assays, the transcriptional start sites of hypothalamic MCH mRNA were determined, allowing us to define the promoter region of this gene. We also characterize the central nervous system distribution of expression of the MCH gene by Northern blot analysis, demonstrating that the MCH mRNA is found predominantly if not exclusively within the hypothalamus.

7. Tyrosinase and other enzymes

- Gershoni-Baruch R, Benderly A, Brandes JM, Gilhar A.
Dopa reaction test in hair bulbs of fetuses and its application to the prenatal diagnosis of albinism. J Am Acad Dermatol 24:220-222, 1991.
Abstract : No information is available on the amount of tyrosinase normally present in fetuses. A dopa reaction test in hair bulbs from the scalp of normal fetuses obtained after abortion showed that tyrosinase is present in fetuses as early as 17 weeks. Only faint activity was detected in skin specimens other than from the scalp. This assay can serve as a quick and reliable method for the prenatal diagnosis of tyrosinase-negative albinism.

- Granholm NH, Opbroek AJ, Harvison GA, Kappenman KE.
Tyrosinase activity (TH, DO, PAGE-defined isozymes) and melanin production in regenerating hairbulb melanocytes of lethal yellow (Ay/a), black (a/a), agouti (AwJ/AwJ/) and albino (a/a/c2J/c2J) mice (C57BL/6J). Pigment Cell Res 3:233-242, 1990.

Abstract : We compared tyrosinase activity (TH, DO, and native PAGE-defined isozymes) and melanin production in particulate and soluble fractions of hairbulb melanocytes of lethal yellow (Ay/a C/C), nonagouti black (a/a C/C), and albino (a/a c2J/c2J) of 3-, 6-, 9-, and 12-day regenerating hairbulbs. With respect to tyrosine hydroxylase (TH) and dopa oxidase (DO) activities, Ay/a melanocytes possessed only 25-35% of the activity of a/a; there were no genotype differences in either the subcellular distribution of activity in soluble and particulate fractions or in the relative increases of activity over the 12-day developmental period. TH data on wild-type agouti (AwJ/AwJ) mice over the 3-11 day regeneration interval showed an activity intermediate between that of a/a and Ay/a; the rate of TH increase reflected black and yellow phases of the agouti hair cycle. Analyses of the number and densities of dopa-sensitive bands following native PAGE of 3-, 6-, 9-, and 12-day hairbulb fractions of a/a and Ay/a mice suggested stage-dependent patterns. A comparison of rates and amounts of melanin production in 3-, 6-, 9-, and 12-day fractions showed consistent melanin production in Ay/a to be 10-20% that of a/a; however, fold increases in melanin production over the four stages were similar between genotypes. Overall, tyrosinase activity data support the notion that agouti locus modification of tyrosinase activity is a graded or quantitative rather than a qualitative phenomenon.

- Jackman MP, Hajnal A, Lerch K.
Albino mutants of Streptomyces glaucescens tyrosinase. Biochem J 274:707-713, 1991.
Abstract : Site-directed mutagenesis was used to determine the functional role of several residues of Streptomyces glaucescens tyrosinase. Replacement of His-37, -53, -193 or -215 by glutamine yields albino phenotypes, as determined by expression on melanin-indicator plates. The purified mutant proteins display no detectable oxy-enzyme and increased Cu lability at the binuclear active site. The carbonyl derivatives of H189Q and H193Q luminesce, with lambda max. displaced more than 25 nm to a longer wavelength compared with native tyrosinase. The remaining histidine mutants display no detectable luminescence. The results are consistent with these histidine residues (together with His-62 and His-189 reported earlier) acting as Cu ligands in the Streptomyces glaucescens enzyme. Conservative substitution of the invariant Asn-190 by glutamine also gives an albino phenotype, no detectable oxy-enzyme and labilization of active-site Cu. The luminescence spectrum of carbonyl-N190Q, however, closely resembles that of the native enzyme under conditions promoting double Cu occupancy of the catalytic site. A critical role for Asn-190 in active-site hydrogen-bonding interactions is proposed.

- King RA, Townsend D, Oetting W, Summers CG, Olds DP, White JG, Spritz RA.
Temperature-sensitive tyrosinase associated with peripheral pigmentation in oculocutaneous albinism. J Clin Invest 87:1046-1053, 1991.
Abstract : Several types of autosomal recessive oculocutaneous albinism (OCA) are associated with abnormal tyrosinase function and a generalized reduction in or absence of cutaneous and eye melanin. Each is thought to result from a different mutant allele at the tyrosinase locus, with the mutation producing an enzyme with little or no activity in all involved tissues. In this paper, we report a new type of OCA that results from a tyrosinase allele producing a temperature-sensitive enzyme. The proband had white hair in the warmer areas (scalp and axilla) and progressively darker hair in the cooler areas (extremities) of her body. Melanocyte and melanosome architecture were normal. Quantitative hairbulb tyrosinase (dopa oxidase) assay demonstrated a loss of activity above 35-37 degrees C. Plasma pheomelanin and urine eumelanin intermediates were reduced and correlated with hair melanin content. This is the first temperature-sensitive tyrosinase mutation to be reported in humans and is analogous to the Siamese mutation in the cat and the Himalayan mutation in the mouse.

- Marwan MM, Jiang JW, de Lauro C.
Psoralens stimulate mouse melanocyte and melanoma tyrosinase activity in the absence of ultraviolet light. Pigment Cell Res 3:214-221, 1990.
Abstract : Psoralens (8-methoxypsoralen, 5-methoxypsoralen and 4,5,8-trimethylpsoralen) stimulate mouse melanoma cell (S91 and B16/F10) tyrosinase activity in vitro in a dose-related manner. Stimulation of enzyme activity by the psoralens was evoked in the presence or absence of light. In the presence of a melanotropin the actions of the psoralens were generally at least additive compared to the individual actions of the two agonists. The actions of the psoralens were acute and depended upon the constant presence of the agents to maintain enhanced melanoma tyrosinase activity. Tyrosinase activation by the psoralens, like

that of alpha-melanotropin, was blocked by actinomycin-D or cycloheximide demonstrating that the actions of the drugs may have involved both transcriptional and translational events in the stimulation of melanogenesis. Psoralens also stimulated an immediate darkening of frog skins in vitro. Topically applied psoralens were transdermally delivered to the systemic circulation resulting in a conversion from pheomelanogenesis to eumelanogenesis within follicular melanocytes throughout the entire skin of mice (C57BL/6JAy maintained in the dark. Taken together, these results demonstrate that psoralens activate processes within melanocytes resulting in both an immediate translocation of melanosomes within the cell (frog) or in a slower genomic event involving tyrosinase activation (melanoma cells) and eumelanin formation (mouse follicular melanocytes).

- Slominski A, Costantino R, Howe J, Moellmann G.
Molecular mechanisms governing melanogenesis in hamster melanomas: relative abundance of tyrosinase and catalase-B (gp 75). Anticancer Res 11:257-262, 1991.
Abstract : Variants of the Bomirski family of hamster melanomas whose proliferative rates differ inversely with the genetically determined degree of melanogenesis were probed for two proteins critical in melanogenesis: tyrosinase and catalase-B (gp 75). The parental black tumor Ma contained both proteins in abundance. The amelanotic variant Ab, inducible in culture with L-tyrosine or L-dopa to form melanosomes and to melanize, had minimal tyrosinase, despite high levels of (tyr)mRNA, and no gp 75. Variant MI, hypomelanotic despite abundant tyrosinase, and synthesizing predominantingly pheo-(red) melanin, expressed barely detectable gp 75. These findings suggest a regulatory control of melanogenesis distal to (tyr)mRNA and strengthen the hypothesis that in vivo tyrosinase without catalase-B favors pheo- over eumelanogenesis.

- Slominski A, Costantino R.
Molecular mechanism of tyrosinase regulation by L-dopa in hamster melanoma cells. Life Sci 48:2075-2079, 1991.
Abstract : Exposure of hamster amelanotic melanoma cells to L-dihydroxyphenylalanine (L-DOPA) resulted in a time dependent increase of cell pigmentation, tyrosinase concentration and activity with peak after 24 hours. Northern blot analysis showed a small but reproducible increase of tyrosinase mRNA after 3 hours and a decrease below the control level after 9 hours. After 24 and 48 hours tyrosinase mRNA was undetectable. It is suggested that L-DOPA or its oxidation products can stimulate intracellular tyrosinase concentration and regulate tyrosinase mRNA level both in positive and negative fashion.

- Slominski A, Costantino R.
L-tyrosine induces tyrosinase expression via a posttranscriptional mechanism. Experientia 47:721-724, 1991.
Abstract : Exposure of hamster amelanotic melanoma cells to L-tyrosine caused a time-dependent increase of tyrosinase protein concentrations, tyrosinase activity and level of cell pigmentation. In contrast, Northern blot analysis using mouse tyrosinase cDNA showed a steady level of tyrosinase mRNA. Thus in hamster melanoma cells the stimulation of intracellular tyrosinase concentration by L-tyrosine is mediated mainly via a posttranscriptional mechanism.

- Taylor AJ.
Two novel assays for tyrosinase and their application to the study of melanoma cell differentiation. Avail. Univ. Microfilms Int., Abstr. Int. B 1991, 51:4288, 1989.

- Tomita Y, Shibahara S, Takeda A, Okinaga S, Matsunaga J, Tagami H.
The monoclonal antibodies TMH-1 and TMH-2 specifically bind to a protein encoded at the murine b-locus, not to the authentic tyrosinase encoded at the c-locus. J Invest Dermatol 96:500-504, 1991.
Abstract : Three hybridomas, TMH-1, TMH-2, and TMH-3, were previously reported by Tomita et al to produce monoclonal antibodies against murine and human T4-tyrosinase localized in melanosome for the formation of melanin pigment. However, TMH antibodies were unable to react with K1735 cells transfected with the authentic tyrosinase-cDNA construct, but did react with those transfected with the pMT4-cDNA construct. The cDNA pMT4 was initially cloned as a putative tyrosinase cDNA by Shibahara et al, but it is now known to encode mouse brown (b) locus protein, which was named "tyrosinase-related protein" by Jackson or "b protein" by Hearing and Jimenez. Furthermore, TMH antibodies recognize hair bulbs of

C57BL/6J-c2J/c2J mouse (B/B, c/c) lacking tyrosinase activity, but do not recognize hair bulbs of b-locus mutated DBA/2 mouse (b/b, C/C), which have authentic tyrosinase. Considering these observations, we conclude that TMH antibodies specifically recognize the protein encoded at b-locus.

8. Melanoma

- Ackerman AB, Sood R, Koenig M.
Primary acquired melanosis of the conjunctiva is melanoma in situ. Mod Pathol 4:253-263, 1991.
Abstract : This essay places the concept of "primary acquired melanosis" of the conjunctiva in historical perspective and shows that it and its analogs, namely, lentigo-melanosis (Hutchinson), melanotic freckle (Hutchinson), melanose circonscrite precancereuse (Dubrueilh), melanotische precancerose (Miescher), lentigo maligna (Clark), precancerous melanosis (Reese), benign, precancerous, and cancerous melanosis (Zimmerman), atypical melanocytic hyperplasia (Silver et al.), and benign acquired melanosis (Zimmerman), are synonyms for melanoma in situ. The issue is not merely semantic or philosophical; it is urgently practical. If a clinician takes literally the meaning of a lesion designated "benign melanosis" and considers it to be benign, rather than the malignant melanoma that it actually is, a patient who bears that flat pigmented lesion may one day die of metastasis from an elevated sequella of it. The same is true of "primary acquired melanosis," which is not simply a condition of blackening by melanin, but a flat melanoma that, if not removed completely, may give rise one day to metastases that cause death. To avoid such misconstructions, we advocate naming melanomas in all organs "melanoma" and those that are confined to epithelial structures "melanoma in situ." Euphemisms like lentigo maligna and primary acquired melanosis are evasions of the diagnosis of melanoma, and use of them may be harmful. For that reason, they should be eschewed.

- Argenyi ZB, Schelper RL, Balogh K.
Pigmented neuroectodermal tumor of infancy. A light microscopic and immunohistochemical study. J Cutan Pathol 18:40-45, 1991.
Abstract : We studied two cases of pigmented neuroectodermal tumor of infancy (PNTI) by routine light microscopy and immunohistochemistry on formalin fixed, paraffin embedded tissues using antibodies to HMB-45 "melanoma associated" antigen, S-100 protein, neuron specific enolase (NSE), Leu-7 antigen, chromogranin, epithelial membrane antigen, collagen Type IV, alpha-fetoprotein and muscle-specific actin and to the intermediate filaments cytokeratin (CK), vimentin, desmin and neural filaments. We found that the large epithelioid cells, many of which contained melanin pigment, were strongly positive for CK and HMB-45, and less intensively positive for vimentin and NSE. The small neuroblast-like cells revealed only focal, weak NSE positivity. Both cell types were negative for S-100 protein and for the other antigens examined. Our results suggest that: (1) the large and small cell populations in PNTI have different immunophenotypes; (2) the expression of CK and HMB-45, together with the S-100 negativity, appears unique for the pigmented cells; and (3) this profile may be helpful in the exclusion of melanoma and peripheral neuroblastoma from the differential diagnosis.

- Angelucci D, Natali PG, Amerio PL, Ramenghi M, Musiani P.
Rapid perinatal growth mimicking malignant transformation in a giant congenital melanocytic nevus. Hum Pathol 22:297-301, 1991.
Abstract : Transformation to malignant melanoma in a giant congenital melanocytic nevus observed on the right limb of a 3,300-g newborn boy was strongly suggested by the histologic features of its ulcerated and papular areas: atypical melanocytes, irregular melanin distribution, many mitotic figures, "pagetoid" invasion of the dermis, and destruction of the rete ridges. Electron microscopy, too, showed that the atypical melanocytes had irregularly shaped and folded nuclei, one or more nucleoli, and a cytoplasm rich in organelles and polymorphous melanosomes. Investigation with a panel of monoclonal antibodies, on the other hand, revealed the antigen phenotype of a proliferative melanocytic lesion unaccompanied by the plain expression of antigens usually observed in malignant melanoma. In addition, the clinical picture during a 2-year follow-up has been free from signs of locoregional and systemic progression.

- Barnhill RL, Mihm MCJ.
Plexiform spindle cell naevus: a distinctive variant of plexiform melanocytic naevus. Histopathology

18:243-247, 1991.
Abstract : Twelve cases of a unique plexiform melanocytic naevus that we have termed plexiform spindle cell naevus are reported. The lesions affected young individuals (mean age 22.5 years) of both sexes and were most frequently located on the shoulders and back. The lesions clinically were slightly raised and blue or darkly pigmented, suggesting blue naevus. Histologically these tumours had a symmetrical wedge-shaped configuration, as seen in typical Spitz naevus, with the apex directed toward the deep reticular dermis or subcutis. The pigmented spindle cells were disposed in fascicles in association with neurovascular bundles and adnexal structures, imparting a plexiform architecture to the lesion. The predominant cell type consisted of spindle cells containing a granular melanin and elongated nuclei. Low-grade cellular atypia was commonly noted. Varying numbers of epithelioid cells were observed in most of the cases. In two cases studied, the naevus cells showed S-100 protein and HMB-45 immunoreactivity. The differential diagnosis of plexiform spindle cell naevus includes malignant melanoma, and spindle and epithelioid cell (Spitz) naevus, blue naevus and combined naevus. Plexiform spindle cell naevus is a distinctive type of pigmented spindle naevus distinguished from the above entities by its striking plexiform architecture, predominance of melanin-containing spindle cells and lack of significant cellular atypia.

- Barnhill RL, Roush GC.
Correlation of clinical and histopathologic features in clinically atypical melanocytic nevi. Cancer 67:3157-3164, 1991.
Abstract : To define better the evolving entity of dysplastic melanocytic nevus (DMN), studies correlating clinical with histologic features of DMN are essential. However, based on a literature search, no previous quantitative analysis was found of the relationship between gross morphologic features and histologic features of DMN. The authors correlated individual clinical features with histopathologic features and histologic diagnosis of the clinically most atypical nevus in 153 melanoma patients. This nevus was identified, evaluated clinically, and removed for histologic evaluation from each patient. Gross morphologic features assessed for nevi included: size (in mm), the presence of a macular component, irregular border, ill-defined border, haphazard coloration, distortion of skin cleavage lines on tangential lighting, asymmetry, and number of colors present (12 features in all). Nineteen histologic features were assessed in each nevus by a single dermatopathologist. These included architectural, nuclear, and cytoplasmic parameters ascribed to dysplastic nevi. Each of these histologic features was correlated with the 12 individual clinical features. Seventeen percent of the nevi fulfilled the criteria for the histologic diagnosis of DMN. Among individual nevus parameters, size (in mm), irregular border, ill-defined border, macular component, and pink color were associated significantly with histologic DMN. Nevus size (in mm) and irregular borders correlated with the greatest number of individual histologic parameters. A comparison of clinicopathologic correlations for two different examiners revealed that certain clinical features are probably more important than others for the recognition of dysplastic nevi and that individual examiners have different thresholds for the perception of some gross morphologic features. These observations are relevant to the development of clinical criteria for dysplastic nevi.

- Beitner H, Nakatani T, Hedblad MA.
A transmission electron microscopical study of dysplastic naevi. Acta Derm Venereol (Stockh) 70:411-416, 1990.
Abstract : In this study those features of naevi that fulfil the clinical and light microscopical criteria of dysplastic naevi have been further examined with transmission electron microscopy. The results have been compared with the structure of normal control skin and compound naevi. In dysplastic naevi most melanosomes were abnormal, with spherical melansomes, an incomplete inner structure and uneven melanin deposit, cigar-shaped melanosomes and macromelanosomes. The intraepidermal border between the nests of dysplastic naevi were uneven and the dysplastic melanocytes extended their cell bodies among surrounding keratinocytes with a tendency to invade the epidermis in an upward direction. These findings will serve as additional criteria for dysplastic naevi.

- Blaustein RL.
Fine-needle aspiration of a metastatic breast carcinoma in the lung with melanin pigmentation: a case report. Diagn Cytopathol 6:364-365, 1990.
Abstract : The presence of melanin pigment in the cytoplasm of breast carcinoma cells has been reported.

Fine-needle aspiration of a solitary lung lesion in a woman who had undergone mastectomy for ductal carcinoma revealed malignant cells consistent with the primary mammary tumor; many of these tumor cells contained melanin pigment.

- Claman LJ, Stetson D, Steinberg B, Shuler CF.
Ultrastructural characteristics of a cell line derived from a melanotic neuroectodermal tumor of infancy. J Oral Pathol Med 20:245-249, 1991.
Abstract : Thin section and freeze-fracture transmission electron microscopy were used to examine and identify the cytoplasmic and membrane structures in a cell line derived from a melanotic neuroectodermal tumor of infancy (MNTI). The cultured cells had a uniform appearance after 70 population doublings characterized by long dendritic processes and evidence of melanin production. The cytoplasm contained numerous melanosomes in various stages of development, vesiculated rough endoplasmic reticulum, microfilaments and uncoated as well as coated vesicles. The membrane specializations included caveoli, coated pits, gap junctions, microfilaments, desmosome-like structures and lamellipodia. The ultrastructural appearance of the cultured MNTI cells was similar to features previously seen in electron micrographs of MNTI tumor specimens. However, correlated freeze-fracture and thin section micrographs permitted further identification of structures previously described. The MNTI cell line represents one of the cell types of the tumor and provides an opportunity for further study of the pathogenesis of this rare tumor.

- Garbe C, Krasagakis K, Zouboulis CC, Schroder K, Kruger S, Stadler R, Orfanos CE.
Antitumor activities of interferon alpha, beta, and gamma and their combinations on human melanoma cells in vitro: changes of proliferation, melanin synthesis, and immunophenotype. J Invest Dermatol 95:231, 1990.
Abstract : The antitumor activities of human interferon (IFN) alpha, beta, and gamma alone or in combination were studied on four human melanoma cell lines (StML-11, StML-12, StML-14, and SKMel-28) in various concentrations (1-50,000 IU/ml IFN alpha, 0.1-1000 IU/ml IFN beta, 1-10,000 IU/ml IFN gamma) in vitro. In all experiments IFN beta exhibited the most potent antiproliferative effect of all IFN tested. After 3 d of incubation a 50% growth inhibition was achieved with 20-40 IU/ml for natural IFN beta and with 600-1200 U/ml for recombinant IFN gamma. Substantially higher doses (7,000 to more than 50,000 IU/ml) of recombinant IFN alpha 2a were required to achieve a 50% growth inhibition. A strong synergistic antiproliferative activity resulted from the combination of IFN alpha with IFN gamma and IFN beta with IFN gamma. None of the IFN tested induced terminal differentiation of melanoma cells in vitro. The formation of dendrites was inhibited, and the portion of differentiated cells in vitro was reduced after treatment with IFN in comparison to the untreated controls (untreated controls: 100%; portion of differentiated cells after treatment with IFN alpha: 58%-74%, IFN beta: 48%-96%, IFN gamma: 10%-33%). The melanin synthesis was slightly elevated after treatment with IFN alpha (untreated controls: 100%; after treatment with IFN alpha: 103%-157%, ns.) and decreased significantly after treatment with IFN beta (49%-71%, p less than 0.05) as well as with IFN gamma (80%-88%, ns.). Cell surface markers were modulated varyingly by the IFN: HLA-I antigens were enhanced by all IFN, with IFN beta emerging as the most potent inducer. Only IFN gamma, however, induced a de novo expression of HLA-DR and -DQ antigens and increased the expression of the ICAM-1 molecule and of the melanoma progression marker A.1.43. Possibly, these findings indicate a biologically more aggressive phenotype of melanoma cells.

- Graham GM, Guarini L, Moulton TA, Datta S, Ferrone S, Giacomini P, Kerbel RS, Fisher PB.
Potentiation of growth suppression and modulation of the antigenic phenotype in human melanoma cells by the combination of recombinant human fibroblast and immune interferons. Cancer Immunol Immunother 32:382-390, 1991.
Abstract : Administration of interferon as a single therapeutic regimen in cancer patients with various neoplasias has had only limited efficacy in ameliorating the negative clinical course of their disease. In the present study, we have evaluated the effect of recombinant human fibroblast (IFN beta) and immune (IFN gamma) interferon, alone and in combination, on growth, differentiation and the expression of class I and II histocompatibility locus antigens (HLA) and melanoma-associated antigens on the human melanoma cell line H0-1. The effect of combinations of interferons on the antigenic profile of human melanoma cells displaying different organ colonization and spontaneous metastatic potential in athymic nude mice was also determined. H0-1 cells were more sensitive to the antiproliferative activity of IFN beta than to IFN gamma and the combination of interferons resulted in a potentiation of growth suppression. The antiproliferative

effect of both interferons was greater in later-passage than in earlier-passage H0-1 cells, possibly reflecting alterations in the evolving tumor cell population as a result of long-term in vitro propagation and/or the selective outgrowth of cells with an increased growth rate. The enhanced growth suppression observed in H0-1 cells treated with the combination of IFN beta plus IFN gamma was not associated with a significant increase in the level of melanin, a marker of melanoma differentiation, above that observed with either interferon used alone. IFN beta and IFN gamma differentially modulated the expression of class I and II HLA and melanoma-associated antigens in H0-1 cells and a series of melanoma cells with different organ colonization and metastatic potential, including MeWo, MeM 50-10, MeM 50-17, 3S5 and 70W. No consistent potentiation or antagonism in the expression of any specific antigen was observed in any of the melanoma cell lines exposed to the combination of interferons. The present study demonstrates that the combination of IFN beta plus IFN gamma can potentiate growth suppression in H0-1 human melanoma cells and that this effect is not associated with an increase in differentiation or a potentiation in antigenic modulation. In addition, no direct correlation between the expression of any specific antigen or its modulation by IFN beta or IFN gamma, alone or in combination, and organ colonization and metastatic potential in nude mice was observed in the different melanoma cell lines.

- Imoto K, Yamazaki Y, Kawahara E, Furukawa M.
Malignant melanocytic schwannoma of the nasopharynx. ORL J Otorhinolaryngol Relat Spec 53:48-51, 1991.
Abstract : A rare case of malignant melanocytic schwannoma in the nasopharynx is presented. Light microscopy showed proliferation of spindle-shaped tumor cells with mitoses. The essential histological hallmark was melanin deposition. Electron-microscopic examination confirmed the schwannian differentiation of the tumor cells. The patient has remained well without evidence of metastasis or local recurrence of the tumor 18 months after the excision of the neoplasm.

- Ishiwata K, Kubota K, Kubota R, Iwata R, Takahashi T, Ido T.
Selective 2-[18F]fluorodopa uptake for melanogenesis in murine metastatic melanomas. J Nucl Med 32:95-101, 1991.
Abstract: The relationship between 3,4-dihydroxy-2-[18F]fluoro-L-phenylalanine (2-[18F]FDOPA) uptake and melanogenesis was studied using mice bearing two B16 melanomas: B16-F1 has a higher melanin synthesis ability and a slower growing rate than the higher metastatic B16-F10. A significantly higher 2-[18F]FDOPA uptake by B16-F1 than by B16-F10 and a reverse relationship for the uptake of [14C] 2-deoxy-2-fluoro-D-glucose and [3H]thymidine were observed 1 hr postinjection. F1-to-F10 ratios of both the 2-[18F]FDOPA uptake and the acid-insoluble radioactivity increased to about 5 at 6 hr, which paralleled the melanin content. FM3A mammary carcinoma showed a 2-[18F]FDOPA uptake similar to the B16-F10 but without the acid-insoluble radioactivity. With D,L-DOPA loading, a 55% decreased uptake by FM3A 1 hr postinjection was significantly greater than the 20% reduction in both melanomas. O-Methylated 2-[18F]FDOPA was a predominant acid-soluble metabolite in all tumors. Whole-body autoradiography discriminated the two melanomas clearly. 2-[18F]FDOPA may be a promising tracer for the selective imaging of melanogenesis.

- Jain AK, Patil S.
Evidence for melanin concentrating hormone (MCH) receptors mediating melanosome aggregation in Labeo melanophores. Indian J Physiol Pharmacol 34:187-190, 1990.
Abstract : Melanin concentrating hormone (MCH: 5 x 10(-12)-5 x 10(-8) M) induced a concentration related, rapid and reversible pigment aggregation in innervated melanophores of Labeo rohita. In inducing melanosome aggregation MCH was found to be 10(4) times more potent than norepinephrine. Experiments employing phentolamine and propranolol suggest that MCH acts through its own specific receptors on the melanophores unrelated to adrenoceptors. MCH was able to aggregate the melanosomes even in the absence of extracellular Ca2+.

- Jara JR, Martinez-Liarte JH, Solano F, Penafiel R.
Transport of L-tyrosine by B16/F10 melanoma cells: the effect of the intracellular content of other amino acids. J Cell Sci 97:479-485, 1990.
Abstract : The uptake of L-Tyr by B16/F10 malignant melanocytes in culture has been studied. These melanoma cells can either be depleted of amino acids by 1 h preincubation in Hanks' isotonic medium or preloaded with a specific amino acid by 1 h preincubation in the same solution containing 2 mM of the

amino acid to be preloaded. By means of these pretreatments, it is shown that the rate of L-Tyr uptake is greatly dependent on the content of other amino acids inside the cells. The L-Tyr uptake is higher in cells preloaded with amino acids transported by the L and ASC systems than in cells depleted of amino acids or preloaded with amino acids transported by the A system. It is concluded that L-Tyr is mainly taken up by an exchange mechanism with other amino acids mediated by the L1 system, although the ASC system can also participate in the process. In agreement with that, the homo-exchange performed by cells preloaded with unlabelled L-Tyr is more efficient than any other hetero-exchange, although L-Dopa, the product of tyrosine hydroxylation in melanin synthesis, is almost as efficient as L-Tyr. Apart from aromatic amino acids, melanoma cells preloaded with L-Met and L-His also yield a high initial rate of L-Tyr uptake. The results herein suggest that melanoma cells do not have transport systems specific for L-Tyr, even if this amino acid is needed to carry out the differential pathway of this type of cells, melanosynthesis.

- Jimbow K, Salopek TG, Dixon WT, Searles GE, Yamada K.
The epidermal melanin unit in the pathophysiology of malignant melanoma. Am J Dermatopathol 13:179-188, 1991.
Abstract : The epidermal melanin unit (EMU) denotes the symbiotic relationship between a melanocyte and a pool of associated keratinocytes. We propose to show that alterations in the biology of the EMU are the main determinant of the different patterns of intraepidermal growth of melanocytes in lentigo maligna melanoma (LMM) and superficial spreading melanoma (SSM). They also appear to affect the biosynthesis of melanin and melanosomes during malignant transformation. Findings in histochemical studies with monoclonal antibodies generated against melanosomal proteins to produce different stains of melanocytes of normal skin, dysplastic melanocytic nevi (DMN), common melanocytic nevi (CMN), LMM, and SSM have led to the suggestion that the altered melanosome synthesis is a main phenotype in the pathophysiology in neoplastic transformation of melanocytes. Altered melanin synthesis may also affect the carcinogenesis in malignant melanoma: pheomelanin is increased in malignant melanoma and DMN, but not in normal skin and CMN. Pheomelanin and its precursors could aid the malignant transformation of melanocytes through the generation of mutagenic ultraviolet photoproducts in familial DMN syndrome.

- Jin KM, Nogita T, Toyoda H, Kawashima M, Hidano A.
Pedunculated pigmented eccrine poroma of the scalp with increased urinary excretion of 5-S-cysteinyldopa. J Dermatol 17:555-558, 1990.
Abstract : We describe a 52-year-old man with a pedunculated pigmented eccrine poroma mimicking a nodular malignant melanoma in the occipital region. The tumor was once resected but soon recurred. Histologically, the tumor mass extended from the epidermis downwards into the dermis and contained melanin granules in some areas. The tumor cells were uniformly cuboidal in appearance and had round, deeply basophilic nuclei. Initially, the urinary excretion level of 5-S-cysteinyldopa (5-S-CD) was high, but, after resection of the tumor, the level of 5-S-CD returned to normal.

- Kageshita T, Nakamura T, Yamada M, Kuriya N, Arao T, Ferrone S.
Differential expression of melanoma associated antigens in acral lentiginous melanoma and in nodular melanoma lesions. Cancer Res **51:1726-1732, 1991.**
Abstract : The reactivity in an avidin-biotin complex immunoperoxidase reaction with a large panel of anti-human melanoma associated antigen (MAA) and anti-HLA monoclonal antibodies of 24 primary and 11 metastatic acral lentiginous melanoma (ALM) lesions was compared to that of 12 primary and 12 metastatic nodular melanoma (NM) lesions. The expression of the membrane bound vitronectin receptor, Mr 110,000 MAA, Mr 97,000 MAA, and intercellular adhesion molecule-1 was significantly lower in both primary and metastatic ALM lesions than in their NM counterparts. Furthermore, primary ALM lesions displayed a significantly lower expression than primary NM lesions of the membrane bound high molecular weight melanoma associated antigen (HMW-MAA), Mr 110,000 MAA, Mr 100,000 MAA, 9-O-acetyl-GD3, GD2-GD3, and GD2, of the cytoplasmic monoclonal antibody 465.12 defined MAA and of transferrin receptor and of HLA-DQ and DP antigens; ALM metastases expressed a significantly lower level of carcinoembryonic antigen-MAA than NM metastases. These antigenic differences do not reflect an antigenic paucity of ALM cells, since ALM lesions express a higher level of T4-tyrosinase than NM lesions and a level of HLA Class I antigens similar to that of NM lesions. In view of the use of HMW-MAA, Mr 97,000 MAA, and GD3 in immunoscintigraphy and/or in immunotherapy, it is noteworthy that the three antigens are expressed in a

similar high percentage of ALM metastases and of primary and metastatic NM lesions, while the HMW-MAA is expressed in a markedly lower percentage of primary ALM lesions than Mr 97,000 MAA and GD3. However, the degree of heterogeneity of HMW-MAA within a positive primary ALM lesion, as measured by the percentage of stained melanoma cells, is lower than that of Mr 97,000 MAA and GD3. The expression of the antigens investigated in ALM and NM lesions was not correlated with the presence of lymphocyte infiltrates, melanin content of melanoma cells, and epithelioid and spindle type of melanoma cells in the lesions. On the other hand, the survival of patients with ALM was inversely correlated with the expression of intercellular adhesion molecule 1 or HMW-MAA in their primary lesions. A potential role of HMW-MAA in the course of the disease is suggested by its significantly higher expression in metastatic than in primary ALM lesions.

- Karg E, Rosengren E, Rorsman H.
Hydrogen peroxide as a mediator of dopac-induced effects on melanoma cells. J Invest Dermatol 96:224-227, 1991.
Abstract : Dopac increases tyrosinase activity and exerts cytotoxic effects in cultures of human melanoma cells. The possible role of hydrogen peroxide in these actions was examined. Catalase (100 micrograms/ml) completely reversed the cytotoxic action of 0.3 mM dopac and reduced its tyrosinase-stimulating effect by approximately one half. The results show that extracellular hydrogen peroxide is a mediator of both the tyrosinase-stimulating and cytotoxic actions of dopac. Analysis of the degradation products of melanin from dopac-treated melanoma cells after hydriodic acid (HI) hydrolysis revealed the presence of aminohydroxy-phenylacetic acid (AHPAc). This substance is obtained by HI hydrolysis of melanin formed by oxidation of cysteinyl-dopac. Thus, the presence of AHPAc indicates that dopac is transported into the melanocytes where it serves as a substrate for tyrosinase.

- Katsuda H, Kobayashi T, Saito H, Matsunaga T, Ikeya M.
Electron spin resonance imaging of mouse B16 melanoma. Chem Pharm Bull (Tokyo) 38:2838-2840, 1990.
Abstract : An X-band electron spin resonance (ESR) imaging apparatus with a pin-hole TE102 mode cavity and a rapid scan coil was constructed. Using this apparatus, ESR imaging of melanin in mouse B16 melanoma was observed for the first time. The ESR spectrum of B16 melanoma is similar to that of natural melanin extracted from sepia officinalis in microwave power dependence.

- Kiguchi K, Collart FR, Henning-Chubb C, Huberman E.
Induction of cell differentiation in melanoma cells by inhibitors of IMP dehydrogenase: altered patterns of IMP dehydrogenase expression and activity. Cell Growth Differ 1:259-270, 1990.
Abstract : To study the induction of differentiation in human melanoma cells, we treated 12 melanoma cell lines with mycophenolic acid and tiazofurin, inhibitors of IMP dehydrogenase (IMPDH). In all cell lines studied, both agents inhibited cell growth and increased melanin content. However, the degree of growth inhibition did not necessarily correspond to the increase in melanin content. A detailed analysis of the HO and SK-MEL-131 cell lines indicated that mycophenolic acid and tiazofurin caused a time- and dose-dependent increase in the expression of a series of other maturation markers, including formation of dendrite-like structures, tyrosinase activity, and reactivity with the CF21 monoclonal antibody. The growth inhibition and melanogenesis induced by the IMPDH inhibitors was abrogated by the addition of exogenous guanosine. No such effect was observed after treatment of the cells with phorbol 12-myristate 13-acetate or retinoic acid, two other inducers of differentiation in these cells. The mycophenolic acid- and tiazofurin-treated cells also showed an increased level of IMPDH mRNA and protein, perhaps because of compensation for the inhibitor-mediated decrease in IMPDH activity. In contrast, treatment with phorbol 12-myristate 13-acetate or retinoic acid resulted in decreased levels of IMPDH mRNA and protein. The lack of a consistent pattern of IMPDH expression in the cells treated with IMPDH inhibitors and phorbol 12-myristate 13-acetate or retinoic acid suggests that the altered expression of IMPDH is not a general requirement for the induction of cell differentiation in these cells. Our results also suggest that IMPDH inhibitors may provide a useful approach to circumvent the differentiation block in melanoma.

- Klein-Szanto A, Bradl M, Porter S, Mintz B.
Melanosis and associated tumors in transgenic mice. Proc Natl Acad Sci USA 88:169-173, 1991.
Abstract : Melanosis was found to various extents in a wide array of tissues of all 23 autopsied mice whose

transgene consisted of the tyrosinase promoter fused to the simian virus 40 early-region oncogenic sequences. Pigmentation in a given animal was attributable to any or all of the following; an increase in numbers of some normally pigmented cells of neural crest origin (a result compatible with early stages of transformation); elicitation of melanin synthesis in some cells that normally have little melanin, or none at all (the latter possibly signaling metaplasia); unusual intercellular transfer of pigment granules from melanocytes into certain normally unpigmented epithelia and endothelia; and profusion of melanin-phagocytizing cells. Neoplasms, occasionally also containing melanin, arose in association with some of these melanotic tissues and included three choroid plexus tumors, three endocardial tumors, two peripheral nerve sheath tumors (schwannomas), two cochlear tumors, two pineal gland tumors, one salivary gland tumor, and one nasal mucosa tumor. These apparently originated independently of the ocular and cutaneous melanomas found in the same animals. The events involved in melanosis may thus contribute to neoplastic conversion.

- Lassmann G, Liermann B, Arnold W, Schwabe K.
Ribonucleotide reductase in melanoma tissue. EPR detection in human amelanotic melanoma and quenching of the tyrosine radical by 4-hydroxyanisole. J Cancer Res Clin Oncol 117:91-95, 1991.
Abstract : The characteristic EPR doublet of tyrosine radicals of the growth-regulating enzyme ribonucleotide reductase was detected in human melanoma tissue grown in nude mice. This was possible through the use of an amelanotic melanoma that does not exhibit disturbing EPR signals from melanin. The content of tyrosine radicals is higher in young tumor tissues than in older ones. The clinically applied antimelanotic drug, 4-hydroxyanisole, inhibits ribonucleotide reductase in Ehrlich ascites tumor cells as demonstrated by a pronounced quenching of tyrosine radicals (IC50 = 5 microM). In amelanotic melanoma tissue tyrosine radicals of the enzyme are also quenched by 4-hydroxyanisole in concentrations down to 50 microM. Thus, the inactivation of ribonucleotide reductase, which provides deoxyribonucleotides for DNA synthesis, may be a hitherto unexpected mechanism for the antitumor action of 4-hydroxyanisole.

- Madoc-Jones H, Wazer DE, Zamenhof RG, Harling OK, Bernard JAJ.
Clinical considerations for neutron capture therapy of brain tumors. Basic Life Sci 54:23-35, 1990.
Abstract : The radiotherapeutic management of primary brain tumors and metastatic melanoma in brain has had disappointing clinical results for many years. Although neutron capture therapy was tried in the United States in the 1950s and 1960s, the results were not as hoped. However, with the newly developed capability to measure boron concentrations in blood and tissue both quickly and accurately, and with the advent of epithermal neutron beams obviating the need for scalp and skull reflection, it should now be possible to mount such a clinical trial of NCT again and avoid serious complications. As a prerequisite, it will be important to demonstrate the differential uptake of boron compound in brain tumor as compared with normal brain and its blood supply. If this can be done, then a trial of boron neutron capture therapy for brain tumors should be feasible. Because boronated phenylalanine has been demonstrated to be preferentially taken up by melanoma cells through the biosynthetic pathway for melanin, there is special interest in a trial of boron neutron capture therapy for metastatic melanoma in brain. Again, the use of an epithermal beam would make this a practical possibility. However, because any epithermal (or thermal) beam must contain a certain contaminating level of gamma rays, and because even a pure neutron beam causes gamma rays to be generated when it interacts with tissue, we think that it is essential to deliver treatments with an epithermal beam for boron neutron capture therapy in fractions in order to minimize the late-effects of low-LET gamma rays in the normal tissue. I look forward to the remainder of this Workshop, which will detail recent progress in the development of epithermal, as well as thermal, beams and new methods for tracking and measuring the uptake of boron in normal and tumor tissues.

- Manabe T, Moriya T, Inagaki Y, Takei Y.
Malignant melanoma and extramammary Paget's disease in the same patient. Am J Dermatopathol 7 Suppl pp. 29-34, 1985.
Abstract : Axillary Paget's disease was found in a 34-year-old man who died of metastatic malignant melanoma. The primary cutaneous malignant melanoma was diagnosed 6 years before the patient's demise and was confirmed by the presence of melanin, a positive dopa reaction, and immunohistochemical demonstration of S-100 protein in neoplastic cells. Postmortem examination revealed Paget's disease of the axillary skin in addition to widespread metastases of malignant melanoma. Paget cells were positive for periodic acid-Schiff reaction and alcian blue. They were also immunohistochemically positive for

carcinoembryonic antigen, but were negative for S-100 protein. This association of malignant melanoma and Paget's disease in the same person has not, to the best of our knowledge, been described before.

- Martinez F, Merenda G, Bedrossian CW.
Lipid-rich metastatic balloon-cell melanoma: diagnosis by a multimodal approach to aspiration biopsy cytology. Diagn Cytopathol 6:427-433, 1990.
Abstract : Fine needle aspirates from one of multiple liver nodules revealed a large number of discohesive malignant cells with abundant vacuolated cytoplasm. The patient had had left eye enucleation the year before, for a melanoma with focal areas of clear cell change. Pap stained preparations from the liver FNA displayed well the nuclear features of balloon cell melanoma, including anisonucleosis, prominent nucleoli and intranuclear inclusions. Diff-Quik stain demonstrated best the cytoplasmic features such as distinct cell margins and finely dispersed and sharply delineated clear vacuoles. No pigmentation was noted but melanoma was suspected after the history prompted comparison with the enucleated specimen resected a year previously. A multimodal battery of ancillary methods including electron microscopy (EM) and immunocytochemistry (ICC) allowed the confirmation of balloon-cell melanoma, a rare variant not previously described in the eye. By EM, abundant lipids were identified along with melanin-containing structures resembling melanosomes. S-100 positivity along with negativity for epithelial, lymphohistiocyte and germ cell markers was compatible with melanoma. These findings are consistent with the view that cytologic detection of poorly cohesive, hypervacuolated cells does not exclude the possibility of melanoma. This rare example of a lipid-containing melanoma stresses the value of obtaining good clinical history, comparing FNAs to any pre-existing material and utiiizing a multimodal approach to cytologic diagnosis in select cases.

- Nilius B, Bohm T, Wohlrab W.
Properties of a potassium-selective ion channel in human melanoma cells. Pflugers Arch 417:269-277, 1990.
Abstract : Currents through ion channels were measured from cells of a human melanin-producing melanoma cell line (IRG 1) with the patch clamp technique. In these cells the most frequently observed channel is a potassium channel. The channel activates slowly at depolarizing voltage steps but does not inactivate. Single channel potassium currents can be measured in cell-attached patches at the resting potential of melanoma cells. The channel has a conductance of approximately 10 pS. As measured from the reversal potentials of single channel currents, the permeability ratio for sodium and potassium, PNa/PK, is between 0.03 and 0.04. Open probability is increased at positive potentials. Mean open times are prolonged at voltage steps to more positive potentials. Closed time histograms are fitted by two exponentials. The slow shut time is decreased at positive potentials. In whole cell measurements, cell conductance measured between -20 and + 70 mV was reduced by 10 mM tetraethylammonium chloride from 6.4 +/- 1.2 nS (n = 4) to 0.8 +/- 0.3 nS (n = 3). Application of isoproterenol decreases the probability of the channel being open without any change in the single channel conductance. A possible role of the 10 pS potassium channel in the growth of melanoma cells is discussed.

- Onoda N, Tsutsumi Y, Kakudo K, Ozawa A, Niizuma K, Ohkido M, Osamura RY.
Pigmented dermatofibrosarcoma protuberans (Bednar tumor). An autopsy case with systemic metastasis. Acta Pathol Jpn 40:935-940, 1990.
Abstract : An autopsy case of pigmented dermatofibrosarcoma protuberans (Bednar tumor) with systemic metastasis is reported. No previous example of this tumor showing widespread metastasis has been reported in the literature. The patient, a 45-year-old man, developed a tumor on the right upper arm. The tumor recurred twice and metastasized to other parts of the skin, lungs and brain during the 8-year clinical course. The primary tumor contained melanin-laden tumor cells and showed a storiform growth pattern. Autopsy confirmed multiple metastatic lesions in the skin, lungs, brain, thyroid, pancreas, stomach, small intestine and thigh muscles. The recurrent and metastatic tumors lacked both melanin production and the storiform arrangement, and instead revealed "fibro-sarcomatous" change with a herring-bone or interlacing pattern of growth.

- Peison B, Benisch B.
Paget's disease of the nipple simulating malignant melanoma in a black woman. Am J Dermatopathol 7 Suppl pp. 165-169, 1985.
Abstract : A lesion of Paget's disease arising in the skin of the nipple in a black woman is reported. The

lesion simulated histologically a malignant melanoma because of the abundance of melanin within neoplastic cells in the epidermis as well as within the underlying ductal carcinoma of the breast. It was only after differential staining that the diagnosis of Paget's disease could be substantiated unequivocally.

- Pettinato G, Manivel JC.
Melanotic neuroectodermal tumor of infancy. A reexamination of a histogenetic problem based on immunohistochemical, flow cytometric, and ultrastructural study of 10 cases. Am J Surg Pathol 15:233-245, 1991.
Abstract : Ten cases of melanotic neuroectodermal tumor of infancy (MNTI) were studied. There were nine males and one female ranging in age from 2 weeks to 10 months; one patient was 8 years old. Sites of origin were the maxilla (five), epididymis (two), mandible (one), skull (one), and soft tissues of the cheek (one). Six tumors recurred from 1 to 18 months after diagnosis. One patient had widespread dissemination. Electron microscopic study of four cases showed cells with melanosomes at various stages of maturation, and cells with neuroblastic features, including neurosecretory granules and cytoplasmic processes. Nine cases of MNTI were studied immunohistochemically. Small neuroblastic cells and large cells in all cases were reactive for neuron-specific enolase (NSE), synaptophysin, HMB45, and dopamine-beta-hydroxylase, large cells in all cases and few small cells were reactive for cytokeratin (CK) and vimentin (VIM). Epithelial membrane antigen was observed in large cells in three cases, four cases expressed Leu 7 antigen, three were focally positive for glial fibrillary acidic protein, one for desmin, and one for chromogranin. All cases were nonreactive for retinol-binding protein, neurofilaments, alpha-fetoprotein, S-100 protein, and carcinoembryonic antigen. Five normal adult retinas were studied similarly; the pigmented epithelium of the retina was reactive for CK, VIM, HMB45, NSE, and S-100. DNA study, performed in eight tumors, revealed aneuploidy in two (DNA index = 1.7 and 1.8); these cases recurred within 1 month. No differences were observed according to site or behavior. MNTI is a primitive neuroectodermal tumor with polyphenotypic expression of neural and epithelial markers, melanin production, occasional glial, and rhabdomyoblastic differentiation, and no photoreceptor differentiation. It probably represents a dysembryogenetic neoplasm that recapitulates the retina at 5 weeks of gestation.

- Shukuwa T, Nonaka S, Yoshida H.
A comparative study of fluorescence in malignant melanoma and nevocellular nevus using a fluorescence microscope and formalin-fixed specimens. J Dermatol 17:538-544, 1990.
Abstract : Fluorescence in malignant melanoma cells was investigated. The specimens from 18 cases of malignant melanoma and 26 cases of nevocellular nevus, which were fixed with formalin and embedded in paraffin wax, were studied by the fluorescence microscopic method. On the fluorescence microscope, the malignant melanoma cells emitted intense fluorescence from the cytoplasm. The nevus cells with large amounts of melanin granules showed moderate fluorescence. The tumor cells of melanoma in situ and nevus cells with few melanin granules emitted little fluorescence. Not only malignant melanoma cells but also nevus cells in the formalin fixed specimens had various degrees of fluorescence. Many cases of malignant melanoma emitted intense fluorescence, but this was rarely found in nevocellular nevus. This method is also useful in differentiating melanoma from nevocellular nevus.

- Shuster S, Huszar M, Geiger B.
Immunofluorescent localization of intermediate filament subunits for the differential diagnosis of malignant melanoma. Am J Dermatopathol 7 Suppl pp. 79-86, 1985.
Abstract : Intermediate filament subunits in normal cells and in their malignant derivatives can be used as specific markers for their histogenetic origins. We have studied five neoplasms of the skin in which positive identification of vimentin containing intermediate filaments by indirect immunofluorescence microscopy helped to establish the diagnosis of malignant melanoma. All of the neoplasms included in this study posed problems in differential diagnosis by conventional light microscopy and yielded equivocal results by conventional histochemistry. Thus, definitive distinction between poorly differentiated carcinoma and poorly differentiated melanoma could not be made by conventional microscopy. In all of the neoplasms described here, immunolabeling with antibodies against different intermediate filaments demonstrated positive staining for vimentin only. This intermediate filament subunit is present in melanocytes (as well as in many mesenchymal cells) but not in epithelial cells. Our study indicates that this technique may be valuable in differential diagnosis of malignant melanoma, particularly in instances where cells lack melanin or show

other atypical morphologic features.

- Szatrowski TP, Nathan CF.
Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51:794-798, 1991.
Abstract : Few nonphagocytic cells are known to generate reactive oxygen intermediates. Based on horseradish peroxidase-dependent, catalase-inhibitable oxidation of fluorescent scopoletin, seven human tumor cell lines constitutively elaborated H2O2 at rates (up to 0.5 nmol/10(4) cells/h) large enough that cumulative amounts at 4 h were comparable to the amount of H2O2 produced by phorbol ester-triggered neutrophils. Superoxide dismutase-inhibitable ferricytochrome c reduction was detectable at much lower rates. H2O2 production was inhibited by diphenyleneiodonium, a flavoprotein binder (concentration producing 50% inhibition, 0.3 microM), and diethyldithiocarbamate, a divalent cation chelator (concentration producing 50% inhibition, 3 microM), but not by cyanide or azide, inhibitors of electron transport, or by agents that inhibit xanthine oxidase, polyamine oxidase, or cytochrome P450. Cytochrome b559, present in human phagocytes and lymphocytes, was undetectable in these tumor cells by a sensitive spectrophotometric method. Mouse fibroblasts transfected with human tyrosinase complementary DNA made melanin, but not H2O2. Constitutive generation of large amounts of reactive oxygen intermediates, if it occurs in vivo, might contribute to the ability of some tumors to mutate, inhibit antiproteases, injure local tissues, and therefore promote tumor heterogeneity, invasion, and metastasis.

- Takahashi H, Parsons PG.
In vitro phenotypic alteration of human melanoma cells induced by differentiating agents: heterogeneous effects on cellular growth and morphology, enzymatic activity, and antigenic expression. Pigment Cell Res 3:223-232, 1990.
Abstract : Sodium butyrate (butyrate), 5-azacytidine (5Aza-C), dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF) were applied to a human melanoma cell line for the purpose of inducing pigmentation and terminal differentiation. The results are summarized as follows: 1) butyrate, DMSO, and DMF had a strong cytostatic effect, arresting cells in the G1 phase of the cycle; 2) butyrate caused a morphological change to spindle shape whereas DMSO and DMF produced rounded cells, without affecting the levels of vimentin and intermediate filaments; 3) tyrosinase activity and melanization were stimulated by DMSO and DMF but not by butyrate; 4) butyrate induced several membrane-bound enzyme activities (alkaline phosphatase and gamma-glutamyl transpeptidase); 5) changes in the expression of antigens related to tyrosinase activity (2B7 and 5C12) only partly corresponded to the changes in enzyme activity; 6) expression of the melanosomal B8G3 antigen was decreased by butyrate, DMSO, and DMF; and 7) the action of DMF resembled that of DMSO whereas 5Aza-C had little effect. The results indicate that these differentiating agents activate different sets of genes, the melanogenic pathway being activated independently of gamma-glutamyltranspeptidase. The down regulation of B8G3 antigen by these agents may provide a common focus for understanding the essential action of differentiation inducers in melanoma cells.

- Tanaka K, Mihara M, Shimao S, Taniguchi K.
The local recurrence of pigmented Spitz nevus after removal. J Dermatol 17:575-580, 1990.
Abstract : A seventeen-month-old female had a pigmented nodule on her left lower leg. The excised lesion was histologically diagnosed as a Spitz nevus, composed mainly of spindle-shaped melanocytes containing large amounts of melanin pigment. When nodular regrowth was seen at the operative site, the recurrent lesion was radically excised as nodular melanoma. However the histological characteristics of the second excised specimen were essentially the same as those in the initially excised one except for the existence of the newly formed collagen fibers, which may suggest an involuting stage in the central portion of the lesion.

- Ucar K.
The effects of histamine H2 receptor antagonists on melanogenesis and cellular proliferation in melanoma cells in culture. Biochem Biophys Res Commun 177:545-550, 1991.
Abstract : B16-C3 murine melanoma, A375P human melanotic melanoma, and C32 human amelanotic melanoma cells were incubated in the presence of (0-4 mM) H2-antagonists, ranitidine and cimetidine. Cell proliferation, tyrosinase activity and melanin content were monitored. H2-antagonists stimulated tyrosinase activity and melanin accumulation in B16-C3 cells in a dose- and time-dependent manner. Stimulation of enzyme activity and pigment production was accompanied by inhibition of cellular proliferation in B16-C3

cells. The inhibitory concentration of cimetidine was approximately 2-fold higher than that of ranitidine. H2-antagonists failed to stimulate melanogenesis in A375P or C32 cells, but inhibited cellular proliferation in both cell lines. These results are the first demonstration of H2-antagonist induced phenotypic changes in malignant melanoma cells in vitro, and represent a novel mechanism for the previously described in vivo antitumor effects of these agents.

- Ueda Y, Kimura A, Kawahara E, Kitagawa H, Nakanishi I.
Malignant melanoma arising in a dermoid cyst of the ovary. Cancer 67:3141-3145, 1991.
Abstract : Autopsy findings of primary malignant melanoma arising in an ovarian dermoid cyst in an 86-year-old woman are presented. The right ovary was replaced by a dermoid cyst, 14 x 9 x 9 cm in size, in which several nodular tumors with diameters less than 3.2 cm were localized. They comprised diffusely proliferating anaplastic cells with prominent nucleoli. Some of them contained melanin pigments in the cytoplasm. The tumor cells were positive for S-100 protein and ultrastructurally showed melanosomes. In addition, several benign pigmented lesions resembling dermal nevus, pigmented schwannoma, or cellular blue nevus were present in the dermoid cyst, one of which contained a malignant melanomatous component. Histologic transition between benign and malignant components and the presence of another small focus of atypical melanocytes in the benign lesion suggested that the malignant melanoma arose in close association with the previously existing benign pigmented lesions in the dermoid cyst.

- Wakamatsu K, Ito S, Fujita K.
Production, circulation, and excretion of melanin-related metabolites in B16 melanoma-bearing mice. Acta Derm Venereol (Stockh) 70:367-372, 1990.
Abstract : Urinary 5-S-cysteinyldopa (5-S-CD) has been used as a biochemical marker of melanoma metastasis. A method was developed for determining the eumelanin-related metabolites 5(6)-hydroxy-6(5)-methyoxyindole-2-carboxylic acids (5H6MI2C and 6H5MI2C) in small volumes of serum. We compared these indoles and 5-S-CD regarding the correlation of their production in melanoma, circulation in blood, and excretion in urine, with the weight of highly pigmented, B16 mouse melanoma. An excellent correlation was found between the serum concentration of 5H6MI2C + 6H5MI2C ($r = 0.92$) and 5-S-CD ($r = 0.89$) and tumor weight. However, the urinary excretion of 5H6MI2C + 6H5MI2C and 5-S-CD did not show any significant correlation. These results suggest that 5H6MI2C + 6H5MI2C and 5-S-CD in serum may better reflect melanoma progression than those in urine. Furthermore, comparison of the contents of these melanin-related metabolites between highly pigmented and less pigmented B16 melanomas suggests that 5-S-CD may be accumulated in pigmented melanoma by virtue of binding to melanin and that catechol-O-methyltransferase (COMT) may play a regulatory role in pigmentation.

- Yamada I, Seki S, Ito S, Suzuki S, Matsubara O, Kasuga T.
The killing effect of 4-S-cysteaminylphenol, a newly synthesised melanin precursor, on B16 melanoma cell lines. Br J Cancer 63:187-190, 1991.
Abstract : We have examined the killing effect of 4-S-cysteaminylphenol (4-S-CAP), a newly synthesised melanin precursor, on B16 melanoma cell lines possessing different melanin-producing activities and found it to be particularly effective in heavily melanised melanoma cells, but less so in moderately melanised melanoma cells, and having no effect on amelanotic melanoma cells and nonmelanoma cells. Thus, it was found that the killing effect of 4-S-CAP is highly dependent upon the synthesis of melanin and tyrosinase in melanoma cells, suggesting that 4-S-CAP may become toxic to melanoma cells only after oxidation by tyrosinase. The killing activity of 4-S-CAP also was found to be associated with a profound inhibition of the thymidine incorporation in pigmented melanoma cells, as compared to the uridine and leucine incorporation. Further, the inhibition of DNA synthesis was most pronounced in heavily melanised melanoma cells, less so in moderately melanised melanoma cells, and not seen in amelanotic melanoma cells. As a possible mechanism that might account for this action, it may be that 4-S-CAP is oxidised by tyrosinase to the o-quinone form via the catechol derivative and that some of the quinones then conjugate with sulfhydryl enzymes including DNA polymerase, thus exerting a killing activity for pigmented melanoma cells. Thus, 4-S-CAP appears to provide a new, effective cytotoxic agent for rational chemotherapy of malignant melanomas.

- Yu DY, Cohen SB, Peyman G, Tso MO.

Mesectodermal leiomyoma of the ciliary body: new evidence for neural crest origin. J Pediatr Ophthalmol Strabismus 27:317-321, 1990.
Abstract : An 8-year-old white boy had a mass of the ciliary body in his left eye. Both the translucent characteristics and the magnetic resonance imaging findings suggested that this lesion was cystic. Fluid aspiration biopsy and iridocyclectomy were performed. By light microscopy the neoplasm showed the characteristic appearance of a neuroid tumor; however, on electron microscopic examination, the tumor exhibited characteristic features of a smooth muscle neoplasm and was diagnosed as a mesectodermal leiomyoma. Observation of melanin granules in scattered tumor cells further confirmed that the tumor had the same origin as uveal melanocytes, which also derive from the neural crest.

9. Eye

- Basu PK, Menon IA, Persad SD, Wiltshire JD.
Binding of chlorpromazine to cultured retinal pigment epithelial cells loaded with melanin. Lens Eye Toxic Res 6:229-240, 1989.
Abstract : The accumulation of chlorpromazine (CPZ) in cultured bovine amelanotic retinal pigment epithelial (RPE) cells artificially loaded with melanin was investigated. The melanin was isolated from human eye bank eyes. Suspensions of the melanin were added to the RPE cells and incubated for 3 hrs. The cells ingested the melanin which was dispersed in the cytoplasm of the cells. They were not adhering to the cell membrane. The melanin-loaded cells grew in culture, although their rate of growth was slower than that of the control RPE cells not loaded with melanin. When the melanin-loaded cells were treated with CPZ, these cells accumulated a greater amount of CPZ than the control cells. A greater amount of CPZ was released from the melanin-loaded cells than from the control cells. The results suggest that some drugs or chemicals such as CPZ could accumulate in vivo in larger quantities and for longer periods in melanotic cells than in nonmelanotic cells. These compounds may subsequently be released into the extracellular fluid, thus affecting the neighbouring cells. This phenomenon may play an important role in the activities of these drugs in the melanotic cells and in the cells adjacent to the melanotic cells. These results suggested that cultured cells loaded with melanin could be used as a suitable model for studying the mechanisms of binding of drugs to intracellular melanin, and of their subsequent release outside the cells.

- Baudouin C, Brignole F, Bayle J, Fredj-Reygrobellet D, Lapalus P, Gastaud P.
Class II histocompatibility antigen expression by cellular components of vitreous and subretinal fluid in proliferative vitreoretinopathy. Invest Ophthalmol Vis Sci 32:2065-2072, 1991.
Abstract : Proliferative vitreoretinopathy (PVR) is the major cause of failure in retinal detachment surgery. It is characterized by the formation of membranes extending along both surfaces of the detached retina and within the vitreous, but the nature of the growing cells has not yet been determined. Using cytologic and immunocytologic procedures with 13 different monoclonal antibodies directed against Class II histocompatibility antigens and various markers of epithelial and immunocompetent cells, 30 specimens were studied of vitreous or subretinal fluid removed from patients with PVR. Five main types of cells could be identified: heavily pigmented cells, poorly pigmented ones, large totally unpigmented macrophage-resembling ones, smaller unpigmented cells, and lymphocytes. Analysis of intravitreal pigment granules, using autofluorescence by epiillumination and cytologic procedures, showed two different populations of pigmented cells: one with autofluorescent lipofuscin granules and the other with exclusively melanin pigment. Immunostaining procedures confirmed the epithelial nonmacrophage lineage of the intravitreal and subretinal cells because most of these cells were positive for cytokeratin but negative for macrophage markers. In addition, 40-100% of these epithelial-derived cells strongly expressed Class II histocompatibility antigens HLA-DR and -DQ. Lymphocytes were found in 13 specimens; B-cells were seen, but no T-lymphocytes could be identified. These results confirm the involvement of retinal pigment epithelial cells and the strong morphologic changes they undergo during the course of PVR. Moreover, the expression of Class II histocompatibility antigens by the growing cells may be related to inflammatory phenomena, but their eventual role in the development and the extension of periretinal proliferation has not been determined.

- Bond JB, Haik BG, Mihara F, Gupta KL.
Magnetic resonance imaging of choroidal melanoma with and without gadolinium contrast enhancement.

Ophthalmology 98:459-466, 1991.
Abstract : Choroidal melanoma is the most common intraocular tumor and is uniquely suited for evaluation by magnetic resonance imaging (MRI) because of the paramagnetic effect of the melanin molecule. The authors performed T1-, T2-, and proton-density-weighted MRI on 34 patients with choroidal melanoma. Nineteen patients received gadolinium contrast, T1-weighted images were superior in both detecting and delineating tumors, showing increased contrast-to-noise ratios over other images. Gadolinium contrast further increased this ratio. These images are presented as evidence that gadolinium-enhanced MRI is valuable in the evaluation of choroidal melanoma.

- Bossard D, Grange JD, Froment JC, Gerard JP, Lyonnet D.
Magnetic resonance imaging in the evaluation of malignant melanoma of the choroid and ciliary body. Bull Soc Ophtalmol Fr 90:865-867, 1990.
Abstract : We evaluated 16 patients with malignant melanomas of the choroid (11) or the ciliary body (5) by magnetic resonance imaging (MRI), using a 0.5 T magnet and a surface coil. The tumor was seen in all cases, hyperintense in T1-weighted images, and hypointense in T2-weighted sequences (80%). These images reflected the short T1 and T2 relaxation times caused by the presence of melanin in those tumors. This non-invasive method was superior to computed tomography (70%), and sometimes superior to fundoscopy (2 cases), angiofluorography (3 cases) or echography (1 case). MRI also proved valuable for differentiating uveal melanoma from associated subretinal effusion.

- Colello RJ, Jeffery G.
Evaluation of the influence of optic stalk melanin on the chiasmatic pathways in the developing rodent visual system. J Comp Neurol 305:304-312, 1991.
Abstract : In a number of mammalian species, fibre outgrowth in the developing retinofugal pathway is coincident with the presence of melanin in the retinal part of the optic stalk. The presence of melanin is transient in this developing system and has been proposed to play a role in the guidance of retinofugal fibres. Further, it has been suggested that this stalk melanin accounts for the differences between the size of the uncrossed retinal component in pigmented and nonpigmented strains. However, a recent study showed that there is no melanin in the optic stalk of Manchester rats during fibre outgrowth. Since such rats supposedly have a normal pigment distribution and a normal pattern of decussation at the optic chiasm, this finding appears to undermine the suggested role played by stalk melanin in establishing the laterality of retinal fibre projections in other mammalian species. The aim of this study was to re-evaluate the relationship between melanin in the stalk and the development of the retinofugal pathway in three strains of rat: the Wild type, Long Evans Hooded, and the Albino. The Albino rat, which lacks melanin-bearing cells entirely, was shown to have the smallest uncrossed projection, approximately 1,340 ipsilaterally projecting cells (ipc), whereas the Long Evans (2,760 ipc) and the Wild-type strain (2425 ipc) were found to have a larger uncrossed retinal component. In both pigmented strains, melanin was restricted to the eye cup and absent from the optic stalk throughout all stages of development.

- Creel DJ, Summers CG, King RA.
Visual anomalies associated with albinism. Ophthalmic Paediatr Genet 11:193-200, 1990.
Abstract : All mammals with hypopigmentation of the retinal pigment epithelium have abnormal visual systems. Albino mammals have been found to have: (1) reduced numbers of uncrossed optic fibers projecting to all visual centers, (2) disorganization of the pattern (lamination) of the dorsal lateral geniculate nuclei, and (3) disorganization of projections from the dorsal lateral geniculate nuclei to the visual cortex. The disorganization of central visual centers has catastrophic effects on stereovision and optokinetic nystagmus. Variable expression in oculocutaneous albinism suggests that affected individuals cannot always be identified by hypopigmentation, reduced visual acuity and nystagmus. Careful observation of foveal development in individuals even with normal vision is necessary to detect all persons with albinism. The scalp-recorded visually evoked potential designed to detect optic misrouting is the most reliable concomitant for determining albinism.

- Cubeddu R, Docchio F, Ramponi R, Boulton M.
Time-resolved fluorescence spectroscopy of the retinal pigment epithelium: age-related studies. IEEE J Quantum Electron 26:2218-2225, 1990.

Abstract : An age-related study of the pigments (melanin, lipofuscin, and melanolipofuscin) of the retinal pigment epithelium, performed by time-resolved fluorescence spectroscopy with picosecond gating, is presented. The study was performed on intact pigment granules, extd. with mild procedures, and the results were correlated with the ultrastructure of the granules and with results of continuous wave absorption and fluorescence spectroscopy. Both classes of pigments exhibit age-related fluorescence properties. These changes may be detected by using continuous wave fluorescence spectroscopy techniques but are greatly enhanced when gating techniques are used. Melanin, in particular, exhibits age-related spectral modifications that suggest a progressive accumulation of lipofuscin-like fluorophores in the granule. The implications of this paper in relation to the possibility of monitoring the fluorescence of these pigments in vivo are discussed.

- Diehl DL, Robin AL, Wand M.
The influence of iris pigmentation on the miotic effect of thymoxamine. Am J Ophthalmol 111:351-355, 1991.
Abstract : We performed a randomly assigned, double-masked, placebo-controlled study in 78 patients with varied iris pigmentation to evaluate the influence of iris pigmentation on the ability of 0.1% thymoxamine to reverse mydriasis produced by 2.5% phenylephrine. Patients were chosen so that a 1.6:1 ratio of dark to light irides was obtained. Within one-half hour after medication, thymoxamine-treated nonbrown irides constricted significantly compared to their fellow placebo-treated irides (P less than .001). Thymoxamine-treated pupils of nonbrown irides were 1.0 to 3.1 mm smaller than placebo-treated fellow eyes. Thymoxamine-treated light brown irides constricted less (0.6 to 2.0 mm) and more slowly compared to fellow placebo-treated irides. Thymoxamine did not reverse the mydriasis in eyes of patients with dark brown irides. Thymoxamine appears similar to other adrenergic agents that bind to melanin, delaying onset and strength of action. Its efficacy as presently formulated may be limited, in part, by iris color.

- Friedman GD, Selby JV, Quesenberry CP, Newman B, King MC.
Eye color and hypertension. Med Hypotheses 33:201-206, 1990.
Abstract : We searched for predictors of essential hypertension in 1,031 persons aged 30-49 who were observed to progress from normotension to hypertension, as compared to an equal number of matched subjects who remained normotensive. Blood pressure status was well documented in both multiphasic screenings and clinical records. Compared to persons with each lighter eye color, those with brown eyes were more prone to develop hypertension, with relative risk of 1.5 (95% confidence interval 1.18-1.96) compared to all persons with nonbrown eyes. The association persisted after control for race, sex, body mass index, alcohol use, educational level, parental history of hypertension, and among whites, for ethnic origin as crudely estimated by last name. Partial confirmation was obtained in three largely independent study groups: 1) 25 pairs of eye-color-discordant dizygotic twins; 2) 894 pairs of incident hypertensives and controls selected only with multiphasic screening blood pressure measurements; and 3) cross-sectional analysis of 152,018 multiphasic screenees. The weak association of eye color with hypertension clearly requires further confirmation. Although it has little potential for use in screening or clinical care, it may have implications regarding etiology. Areas for further exploration include the close metabolic relation of melanins to catecholamines, both derived from the amino acid tyrosine, and the possibility that dark-eyed persons react more quickly and strongly to stimuli than light-eyed persons.

- Fukuda M, Sasaki K.
Intraocular dynamic mode differences of new quinolone antibacterial agents between pigmented and albino rabbit eyes. Lens Eye Toxic Res 6:339-351, 1989.
Abstract : The influence of melanin on the intraocular dynamics of a new quinolone anti-bacterial agent, NY-198, was investigated in albino and pigmented rabbit eyes. Drug uptake into the cornea of the removed eye was almost the same in both albino and pigmented eyes. However, drug uptake into the iris-ciliary body and release volume and time from the tissues were significantly higher and longer in pigmented eyes than in albino rabbit eyes. Penetration of NY-198 into the cornea, the iris-ciliary body and the serum, administered either systemically or locally into the living eyes of pigmented rabbit was significantly higher than that observed in albino rabbit eyes. Drug affinity for melanin was examined utilizing synthetic melanin. Regarding OFLX, NY-198, CEZ, LMOX, CMX, SISO and TOB, drug-melanin combined ratios ranged from 9.1% to 95.5%. SISO and TOB showed antibacterial activity reduction against E, Coli and B. Subtilis. The results suggest that melanin influences the intraocular dynamic mode of a new quinolone agent, NY-198, and that useful information about the influence of melanin in the drug dynamics of ocular tissues can be obtained from in

vitro experiments.

- Loffler KU.
Iris melanocytes on the posterior corneal surface in congenital glaucoma. A clinicopathological case report. Cornea 10:70-74, 1991.
Abstract : The histological and ultrastructural pathology of two corneas that were removed from an infant with congenital glaucoma prior to keratoplasty is described. Clinically, the glaucoma was suspected to be due to an inapparent congenital viral infection, although there was no serological proof. Macroscopic and light microscopic examination of the posterior corneal surface revealed an irregular network of heavily pigmented cells that were overlying an incomplete endothelium and a grossly thickened Descemet's membrane. By scanning and transmission electron microscopy, these melanin-laden cells exhibited the features of iris stromal melanocytes. No evidence of viral infection could be found. The possible mechanisms leading to this unusual morphology are considered and will be discussed in relation to current theories on the etiology of congenital glaucoma.

- Magomedov NM, Dzhafarov AI, Iusifov EI.
The role of melanin in regulating free radical processes in the epithelial eye pigment in acute hypoxia. Biofizika 35:977-980, 1990.
Abstract : Retinal response to the acute hypoxia and reoxygenation were shown to be independent of melanin presence in the eye pigmental epithelium (PE), i.e. in the retinas of both pigmented animals and albinos hypoxia survived an increase in the content of hydroperoxidases and malonyl dialdehyde; subsequent reoxygenation resulted in a more considerable accumulation of lipid peroxidation (LPO) products in both retinal types. Nevertheless under reoxygenation the number of malonyl dealdehyde became below the norm in PE of melanin-containing animals, but not in the albinos. At the same time acute hypoxia increased by 31% the electron spin resonance (ESR) uptake in the pigmented animals. Subsequent reoxygenation inconsiderably suppressed the intensity of the ESR signal. Under the above conditions pretreatment with Na2SeO3 increased the intensity of PE ESR uptake more than 2.5 times with following retrieval to the control level at the 20th minute of reoxygenation. We suggest that under hypoxia with subsequent reoxygenation the melano-protein granules favour regulation of LPO in PE, but not in other retinal layers, the findings being considerably different from those related to potent light effect.

- Pigeau I, Legeais JM, D'Hermies F, Fayet B, Leport M, Abenhaim A, Guinet C, Levy C, Renard G, Vadrot D.
Rapid imaging in orbito-ocular pathology. Contribution of gadolinium. J Fr Ophtalmol 13:285-292, 1990.
Abstract : To evaluate Gradient-Echo Imaging (GEI) in orbito-ocular pathology, 15 volunteers and 34 patients (40 lesions) were examined with GEA T1 and GEA T2 (0.5 T). Results were compared with SE T1 in all cases, with SE T2 in 20 cases and with other imaging modalities (CT). 30 patients were examined before and after injection of gadolinium. Final diagnosis was obtained by surgery or biopsy in 24 cases or by combined results of imaging and clinical findings in 16 cases. Compared with SE, GEA demonstrated a better visualisation of optic nerve, orbital muscles, choroidal-retinal layer, lens capsule and episclera and a better detection of small lesions. It is very helpful for characterisation of lesions containing hemorrhages or paramagnetic components (melanine, gadolinium) or of vascular nature (angioma). Gadolinium was useful for detection of small lesions or characterisation of a few lesions. Thus GEA seems to be an efficient method for the evaluation of orbito-ocular pathology.

- Salceda R, Riesgo-Escovar JR.
Characterization of calcium uptake in chick retinal pigment epithelium. Pigment Cell Res 3:141-145, 1990.
Abstract : 45Ca uptake was studied in isolated chick retinal pigment epithelial cells. 45Ca was accumulated by a saturable, temperature-dependent system with a KM of 400 microM and a Vmax of 0.13 mumoles2mg protein/min, which depends on the external sodium concentrations. The transport system was present early during embryonic development. RPE cells of three breeds of chicks with different degrees of pigmentation accumulated calcium proportionally to the melanin content of the cells, suggesting that pigment granules participate in the storage and regulation of intracellular calcium.

- Smith BS, Walker NJ.
Ultrastructure of murine trisomy 1 optic cup. Teratology 42:581-591, 1990.

Abstract : The optic cups of two gestational day 11 trisomy (ts) 1 mouse embryos and a normal littermate control were examined using transmission electron microscopy (TEM). One trisomic embryo had a small lens with a lens stalk; the other was aphakic. The resolution available with TEM allowed detailed evaluation of cell organelles, spatial relationships, and the intra- and extracellular structural environment of the optic cup in normal and abnormal mouse embryos. Differences between the normal littermate and the trisomic optic cups, as well as between the two ts 1 structures, included the following: 1) melanin granules in the retinal layer and intraretinal space as well as in the pigment layer, 2) neither pseudostratified nor cuboidal neuroepithelium in trisomic optic cups, 3) increasing cell lysis with severity of eye defect, 4) fusion between retinal and pigment layer cells and cells from the pigment layer and head mesoderm. This investigation not only confirmed some of the abnormal morphology found in light microscopic studies of ts 1 at this gestational age but also identified other anomalies in the ts 1 eye that may play a part in the dysgenesis of this organ. The roles of larger than normal intercellular lacunae, disorganized microtubules, and the connections between different cell types in the ts 1 optic cup require further investigation.

10. Other

- Aliev GA, Ivanov AV, Rachkovskii ML, Davydovskaya NG.
EPR spectroscopic study of melanosomes isolated from sheep wool of different color genotypes. Dokl Akad Nauk SSSR 315:1396-1400, 1990.
Abstract : EPR spectroscopic investigations were carried out on melanosomes isolated from sheep wool of various breeds and color genotypes with the aim of revealing the differences in the structural organization of the melanosomes of different genotypes. For example, the specific content of paramagnetic centers in melanin from pale-yellow wool was 4.5-fold lower than in melanin from black wool. The content of melanin in the wool was variable even in similar colored wool, a factor affecting the interpretation of the results.

- Barr-Hamilton RM, Matheson LM, Keay DG.
Ototoxicity of cis-platinum and its relationship to eye colour. J Laryngol Otol 105:7-11, 1991.
Abstract : The following hypothesis is presented: that the susceptibility of an individual patient to hearing loss as a result of cis-platinum administration can be predicted on the basis of eye colour. The rationale is that the melanin content of the inner ears is related to that of the eyes; dark eyes contain more melanin than light-coloured eyes; and melanin causes the accumulation of the ototoxic drug within the inner ear. Hence those with dark eyes will suffer greater damage to the hearing than those with pale eyes. An investigation that confirmed this hypothesis is reported. In addition to cochlear damage there is a significant likelihood of damage to the auditory nerve as a result of the treatment.

- Brazzelli V, Borroni G, Dal T.
Amiodarone-induced pigmentation. A histological, ultrastructural study and review of the literature. G Ital Dermatol Venereol 125:521-526, 1990.
Abstract : Amiodarone is an iodinated cardiac antiarrhythmic drug that causes a slate- gray discoloration of sun-exposed skin. Histopathologically, biopsy specimens of two patients affected by amiodarone pigmentations reveal yellow-brown granules in the reticular dermis, both in the cytoplasm of macrophages and between the collagen bundles. The histochemical stainings of the granules suggest that a lipofuscin pigment rather than melanin is present in the granules. Electron microscopy displays distinctive intracytoplasmic inclusions in many dermal cell types. Six morphologic types can be seen: 1) electron-lucent, membrane bound granules, 2) granules with electron dense nucleus, 3) lamellar "myelin-like" granules, 4) granules with a combination of electron-dense and electron-lucent areas, 5) electron-dense membrane-bound granules, 6) electron-dense no-membrane granules. The different dimensions, structure and shape are related to the structural and aggregational phases of the granules. In particular their pathogenesis may be related to the action of the drug on cell membranes with thesaurismosis, local metabolic damage, accumulation of the drug in the lysosomes and acceleration of the physiological ageing cell process.

- Bridelli MG, Crippa PR, Ugozzoli F.
X-ray diffraction studies on melanins in lyophylized melanosomes. Pigment Cell Res 3:187-191, 1990.
Abstract : A series of experiments was performed on lyophylized melanosomes in order to analyze the

melanin in the natural state as polymerized into these organelles and to verify in such biological amorphous material the possibility of obtaining intensity scattering curves from which Bragg distances can be calculated. The results confirm the feasibility of the method and show that melanins in melanosomes maintain many structural features of the purified form and that the biochemical composition of the organelles can be responsible for the observed differences in the diffractograms. The presence in melanosomes of supramolecular paracrystalline aggregates was also clearly demonstrated.

- Cobb LM, Nolan J, Butler SA.
Distribution of pimonidazole and RSU 1069 in tumour and normal tissues. Br J Cancer 62:915-918, 1990.
Abstract : The tritium-labelled analogues of pimonidazole and RSU 1069 were injected into mice bearing the KHT murine sarcoma which has a hypoxic cell fraction of approximately 10%. The distribution of activity at 24h was recorded using autoradiography and measurement of tissue activity. Autoradiographs with both drugs showed high activity in particular cells within tumour, eye (melanin-associated cells), eyelid (Meibomian gland), liver (centrilobular area), skin (sebaceous gland and melanin), stomach (squamous area), footpad, oesophagus, labial gland, Zymbal's gland, preputial gland, parotid gland (intralobular ducts) and airway epithelium. These tissues had previously been identified as sites of binding of misonidazole. The measurement of total tissue radioactivity showed significantly higher activity in liver, eyelid (Meibomian gland), oesophageal lining, kidney and labial gland than was found in the tumour.

- De Boeck K, Van Cauter A, Fivez H, Smet M, Eeckels R.
Percutaneous drainage of lung abscess in a malnourished child. Pediatr Infect Dis J 10:163-164, 1991.

- Imae K, Kamachi H, Yamashita H, Okita T, Okuyama S, Tsuno T, Yamasaki T, Sawada Y, Ohbayashi M, Naito T.
Synthesis, stereochemistry, and biological properties of the depigmenting agents, melanostatin, feldamycin and analogs. J Antibiot (Tokyo) 44:76-85, 1991.
Abstract : Syntheses of melanostatin and feldamycin have been completed from L-serine and L-threonine, respectively, and the configuration of unknown asymmetric carbons determined. Feldamycin analogs have also been prepared and the L-tryptophyl analog was the most potent in the depigmentation of Streptomyces bikiniensis and B16 melanoma cells.

- Montefiori DC, Zhou JY.
Selective antiviral activity of synthetic soluble L-tyrosine and L-dopa melanins against human immunodeficiency virus in vitro. Antiviral Res 15:11-25, 1991.
Abstract : Melanins are pigments found in hair, skin, irides of the eye, and brain. Their functions in mammals include protection from exposure to sunlight, camouflage from predators, sexual recognition within species, and possible electron transfer reactants. Most natural melanins exist in an insoluble form, which is one reason there is little information on the biological properties of soluble melanins. Here, synthetic soluble melanins were obtained by chemical oxidation of L-tyrosine or spontaneous oxidation of L-beta-3,4-dihydroxyphenylalanine (L-dopa). Replication of human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) was inhibited by soluble melanin in two human lymphoblastoid cell lines (MT-2 and H9) and in phytohemagglutinin-stimulated human T cells. Effective concentrations of 0.15-10 micrograms/ml had no cell toxicity. Melanin blocked infection by cell-free virus and interfered with HIV-induced syncytium formation and cytopathic effects when fusion-susceptible, uninfected cells, were mixed with chronically infected cells. Melanin also impeded the HIV-1 envelope surface glycoprotein, and T cell specific monoclonal antibody leu-3a (CD4), but not leu-5b (CD2), from binding to the surface of MT-2 cells. No effect on HIV-1 reverse transcriptase activity in viral lysates was observed. These results identify a unique biological property of melanin, and suggest that soluble melanins may represent a new class of pharmacologically active substances which should be further investigated for potential therapeutic utility in the treatment of Acquired Immune Deficiency Syndrome (AIDS).

- Wong M, Jimbow K.
Selective cytotoxicity of N-acetyl-4-S-cysteaminylphenol on follicular melanocytes of black mice. Br J Dermatol 124:56-61, 1991.
Abstract : Previous in vivo studies have shown that 4-S-cysteaminylphenol (4-S-CAP) and N-acetyl-4-S-

cysteaminylphenol (N-Ac-4-S-CAP) have antimelanoma effects and that N-Ac-4-S-CAP produced a 98% depigmentation of hair follicles of black mice. This study investigated the process of selective melanocytotoxicity by N-Ac-4-S-CAP through light and electron microscopy studies of hair follicles obtained from newborn black mice treated with N-Ac-4-S-CAP. Visible changes in follicular melanocytes were found 4 h after intraperitoneal (i.p.) administration. Clumps of melanin granules and areas of melanocytic nuclear condensation were seen in the hair follicles. On electron microscopy there was progressive destruction of melanocytes with swelling of membranous organelles, nuclear condensation, and vacuolation of the cytoplasm, culminating in completely necrotic cells. None of these changes were demonstrated in the surrounding keratinocytes. N-Ac-4-S-CAP appears to have specific, cytotoxic effects on melanocytes actively producing eumelanin. The drug may not affect precursor or dormant melanocytes which retain the ability to become active, melanin-producing cells.

- Yamazaki K, Ohmori T, Kumagai Y, Makuuchi H, Eyden B.
Ultrastructure of oesophageal melanocytosis. Virchows Arch A Pathol Anat Histopathol 418:515-522, 1991.
Abstract : Four examples of an endoscopically detected oesophageal melanotic lesion were examined by light microscopy, light microscope histochemistry and transmission electron microscopy, and were compared with 13 control samples of normal oesophageal epithelium. By light microscopy, pigmented melanocytes lacking atypia and mitoses were observed amongst the keratinocytes in the basal layer of the oesophageal mucosa. Junctional activity was absent. The mechanism of pigmentation was studied and found to consist of: an increase in the number of melanocytes in the basal layer of the mucosa, an increase in the quantity of melanin in these melanocytes, transfer of melanin from melanocytes to keratinocytes and to macrophages and fibroblasts in the tunica propria. Since all the lesions demonstrated increased numbers of both melanocytes and melanosomes, the term oesophageal melanocytosis rather than melanosis is suggested, to emphasise the essential character of the lesion as a cellular proliferation. The value of sampling these pigmented lesions during endoscopy is emphasised as a means of obtaining well-preserved material for the evaluation of a lesion which some authorities have viewed as a possible precursor for oesophageal malignant melanoma.

- Youdim MB, Ben-Shachar D, Riederer P.
The role of monoamine oxidase, iron-melanin interaction, and intracellular calcium in Parkinson's disease. J Neural Transm Suppl 32:239-248, 1990.
Abstract : Recent evidence suggests that iron accumulates in substantia nigra pars compacta of patients with Parkinson's disease (PD). This finding is compatible with changes in the respiratory chain activity, increase of malondialdehyde concentration (a measure of lipid peroxidation), decrease of enzyme activity of enzymes involved in detoxication of hydrogen peroxide and oxygen radical species, increased MAO-B-activity in this brain area etc. All these data suggest that oxidative stress may play a certain role in the pathobiochemistry of PD. In addition to the description of the neuroprotective mechanism of the MAO-B-inhibitor L-deprenyl a new aspect focuses the role of the endogenous MAO-B substrates "polyamines" which occur both in neurons and glia. A further aspect of this review deals with the role of calcium as cellular toxin. Although of major importance it is not decided yet whether these biochemical changes are primary or secondary importance to the pathogenesis of PD.

- Zelei BV, Walker CJ, Sawada GA, Kawabe TT, Knight KA, Buhl AE, Johnson GA, Diani AR.
Immunohistochemical and autoradiographic findings suggest that minoxidil is not localized in specific cells of vibrissa, pelage, or scalp follicles. Cell Tissue Res 262:407-413, 1990.
Abstract : Immunohistochemistry with a minoxidil antibody suggested that minoxidil-immunoreactivity is associated with the root sheaths, laterally orientated differentiating matrix cells, and dividing epithelial cells of cultured vibrissa follicles of pigmented and albino neonatal mice. The dermal papilla and connective tissue sheath were devoid of minoxidi-immunoreactivity. To verify that minoxodil-immunoreactivity in the follicles was specific, immunostaining was conducted with dissected whisker pads, formalin-fixed "dead" follicles, and sections of spleen, liver and kidney (non-haired organs) cultured with minoxidil. Microscopic examination revealed minoxidil-immunoreactivity in all of these tissues. Follicles and whisker pads cultured with minoxidil, then washed for one h in media were devoid of minoxidil-immunoreactivity. These data suggest that minoxidil-immunoreactivity in cultured vibrissa follicles is probably non-specific. Sections of skin from C3H and CF1 mice which were topically dosed with minoxidil (in vivo) showed no minoxidil-

immunoreactivity. Autoradiography demonstrated that tritiated minoxidil was bound in vivo and in vitro only to melanin granules in pigmented follicles of rodent and human tissue. This is probably non-specific binding since melanin is known to accumulate several chemically and pharmacologically unrelated drugs. It is reasonable to conclude that, under the conditions of these experiments, minoxidil is not specifically localized in any cells of whisker, pelage or, scalp follicles.

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G. GHANEM
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Laboratory of Oncology and Experimental Surgery
Institut Jules Bordet
Rue Héger-Bordet 1
B - 1000 Brussels

NEWS FROM THE ESPCR

Prof. ALLERGI G.
University of Padova
Dept of Pharmaceutical Sciences
Via Mazzolo 5
I - 35100 PADOVA

Dr. AMILCARELLI F.
University of l'Aquila
Dept of Cell Physiology
Via Assergi 6
I - 67100 L'AQUILA

Prof. ANDERS F.
Genetisches Institut der
Justus Liebig Universitat
Heinrich Buff Ring 58-62
D - 6300 GIESSEN

Dr. ANDREASSI L.
Universita di Siena
Clinica Dermatologica
Piazza Duomo 2
I - 53100 SIENA

Dr. AQUARON R.
Faculte de Medecine
Labo. de Biochimie Medicale
Bld Jean Moulin 27
F - 13385 MARSEILLE CEDEX 5

Dr. ARSTRAND K.
University Hospital
Dept of Clinical Chemistry
S - 581 85 LINKOPING

Dr. AUBERT C.
Lab. Recherches sur les Cancers
Cutanes et la Pigmentation
INSERM U.119
Bld Lei Roure 27
F - 13009 MARSEILLE

Dr. AUGUSTSSON A.
Sahlegrens Hospital
Dept Dermatology Venereology
Karl-Gustavsgatan 36
S - 411 31 GOTHENBURG

Prof. BAGNARA Joseph T.
Health Sciences Center
Dept of Anatomy
1501 N Campbell Avenue
Tucson, Arizona 85721
UNITED STATES OF AMERICA

Dr. BARRENAS M-L.
Dept Occupational Noise
Roda Straket 12
Sahlgrenska Sjn
S - 413 45 GOTHENBURG

Dr. BARTELT R.N.
Praunheimerweg 25
D - 6 FRANKFURT/MAIN 50

Dr. BENATHAN M.
CHUV
Dept of Dermatology
CH - 1011 LAUSANNE

Dr. BERGMAN W.
University Medical Center
Dept of Dermatology
Rijnsburgerweg 10
NL - 2300 RC LEIDEN

Dr. BERTHIER-VERGNES O.
Centre Leon Berard
Rue Laennec 28
F - 69373 LYON CEDEX 08

Dr. BIASOLO M.
Dipto Scienze Farmaceutiche
Universita di Padova
Via Mazzolo 5
I - 35100 PADOVA

Dr. BIRCH M.
Imperial Cancer Research Fund
Biology & Metastasis Lab.
Lincoln's Inn Fields
UK - LONDON WC2A 3PX

Dr. BLEEHEN S.S.
Royal Hallamshire Hospital
Dept of Dermatology
UK - SHEFFIELD S10 2JF

Mr. BOORMAN G.C.
Stiefel Int. Division
Clinical Research Dept.
Holtspur Lane
Wooburn Green
UK - BUCKS HP10 0AU

Dr. BOROVANSKY J.
Dept of Biochemistry
Charles University
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr. BOTTI D.
University of l'Aquila
Dept of Cell Physiology
Via Assergi 6
I - 67100 L'AQUILA

Mr. BOUE-GRABOT M.
Biogir S.A.
Z.I. de Toctoucau
F - 33610 CESTAS

Prof. BOWERS R.R.
Dept of Biology
California State University
5151 State University Drive Center
Los Angeles, CA 90032
UNITED STATES OF AMERICA

Prof. BREATHNACH A.S.
Division of Anatomy, UMDS
St Thomas's Campus
St Thomas Hospital Med. School
Lambeth Palace Road
UK - LONDON SE1 7EH

Dr. BRIDELLI M.G.
University of Parma
Dept of Physics
Via delle Scienze
I - 43100 PARMA

Dr. BROOKS G.
Imperial Cancer Research Fund
Biology & Metastasis Lab.
Lincoln's Inn Fields
UK - LONDON WC2A 3PX

Dr. BRUFAU C.
Avenue Juan Carlos I, 6, 7H
30008 Murcia
SPAIN

Dr. CALDERINI G.
FIDIA s.p.a.
Via Ponte della Fabbrica 3/A
I - 35031 ABANO TERME (PD)

Dr. CASCINELLI Natale
Istituto dei Tumori
Via Venezian 1
I - 20133 MILANO

Dr. CAUSSE C.
l'Oreal
Av. Eugene Schueller 1
F - 93600 AULNAY SOUS BOIS

Dr. CESARINI Jean-Pierre
Fondation Rotschild
Rue Manin 25
F - 75019 PARIS

Dr. CICERO R.
Ist di Biologia Generale
Policlinico
Piazza Giulio Cesare
I - 70124 BARI

Mr. COOKSEY C.L.
University College London
Dept of Chemistry
Gower Street
UK - LONDON WC1H 0AJ

Mrs. CORSARO C.
University of Catania
Dept of General Biology
Via Androne 81
I - CATANIA, SICILIA

Dr. CRESCENZI O.
University of Naples
Dept Organic & Biol. Chemistry
Via Mezzocannone 16
I - 80134 NAPOLI

Prof. CRIPPA P.R.
University of Parma
Dept of Physics
Via delle Scienze
I - 43100 PARMA

Dr. DE WOLFF-ROUENDAAL, Dr.
Academic Hospital
Dept of Ophtalmology
PO Box 9600
NL - 2300 RC LEIDEN

Dr. DEFLANDRE A.
l'Oreal
Av. Eugene Schueller 1
F - 93600 AULNAY SOUS BOIS

Dr. DEL PORTO
Cattedra di Genetica Medica
c/o Ospedale L. Spallanzani
Via Portuense 292
I - 00149 ROMA

Prof. DORE Jean-François
Centre Leon Berard
Laboratoire d'Immunologie
Rue Laennec 28
F - 69373 LYON CEDEX 08

Prof. DUCHON J.
Dept of Biochemistry
Charles University
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr. DUMAS M.
LVMH Recherches
Rue de Seine 50
F - 92700 COLOMBES

Dr. EBERLE Alex
Dept of Research (ZLF)
University Hospital
Hebelstrasse 20
CH - 4031 BASEL

Mr. EDWARDSON J.
Elm Bank Close
Leamington Spa
UK - WARWICKSHIRE CV32 6LR

Prof. EISNER M.
University of Houston
Dept of Physics
4000 Calhoun Road
Houston, Texas 77004
UNITED STATES OF AMERICA

Dr. FERRER C.
Dept of Cell Biology
School of Medicine
Murcia 30100
SPAIN

Dr. FORLOT P.
Laboratoires Goupil s.a.
Avenue du President Wilson 30
F - 94320 CACHAN

Dr. FOURTANIER A.
L'Oreal
Av. Eugene Schueller 1
F - 93600 AULNAY SOUS BOIS

Prof. FRENK E.
University of Lausanne
Dept of Dermatology
CH - 1011 LAUSANNE

Dr. FRIEDMANN P.
Royal Victoria Infirmary
Dept of Dermatology
UK-NEWCASTLE UPON TYNE NE1 4LP

Prof. FRITSCH P.
University of Innsbruck
Dept of Dermatology
Anichstrasse 35
A - 6020 INNSBRUCK

Dr. GALLONE A.
Isto Biologia Generale
Policlinico
Piazza Giulio Cesare
I - 70124 BARI

Dr. GAUTHIER Y.
Cours de Luze 75
F - 33300 BORDEAUX

Dr. GEREMIA E.
Istituto Biologia Generale
Via Androne 81
I - 95124 CATANIA

Dr. GHANEM Ghanem
Lab. Oncol. Exp. Surgery
Universite Libre de Bruxelles
Institut Jules Bordet
Rue Heger-Bordet 1
B - 1000 BRUXELLES

Dr. GIACOMONI P.
L'Oreal
Av. Eugene Schueller 1
F - 93600 AULNAY SOUS BOIS

Dr. GIANNOTTI Benvenuto
Clinica Dermatologica II
Via della Pergola 58
I - 50121 FIRENZE

Dr. GRAMMATICO P.
Cattedra di Genetica Medica
c/o Ospedale Lazzaro Spallanzani
Via Portuense 292
I - 00149 ROMA

Dr. GREEN A.
Queensland Inst. Med. Res.
Dept of Epidemiology
Bramston Terrace
Herston, Brisbane, QLD 4029
AUSTRALIA

Dr. GUARNERI B.
Clinica Dermatologica
Policlinico
Universitario - Gazzi
I - 98100 MESSINA

Dr. HADLEY M.E.
Dept of Anatomy
University of Arizona
Arizona Health Sciences Center
Tucson, Arizona 85724
UNITED STATES OF AMERICA

Dr. HANSSON C.
University Hospital
Dept of Dermatology
S - 221 85 LUND

Dr. HEARING E.D.
Data Abstract Search Service
2477 Regina Drive
Clarksburg, MD 20871
UNITED STATES OF AMERICA

Dr. HEARING Vincent
N.I.H.
Laboratory Cell Biology
Bldg 37, Room 1B22
Bethesda, MD 20892
UNITED STATES OF AMERICA

Dr. HEDIN A.
Centrallasarettet
Dept of Periodontology
S - 631 88 ESKILSTUNA

Dr. HILL H.Z.
New Jersey Medical School
MSB-E586
South Orange Avenue 185
Newark NJ 07103-2757
UNITED STATES OF AMERICA

Prof. HOLL A.
Inst General Special Zoology
Justus Liebig Universitat
Heinrich Buff Ring 38/MZVG
D - 6300 GIESSEN

Dr. HORNBY J.E.
Reading University
Dept Pure & Applied Zoology
UK - READING RG1 5DP

Prof. HUNTER J.A.A.
The Royal Infirmary
Dept of Dermatology
Level 4, Phase 1 building
UK - EDINBURGH EH3 9YM

Dr. JOSEPH D.N.
Stiefel Laboratories
Wooburn Green
High Wycombe
UK - BUCKS HP10 0AU

Prof. JUNG E.G.
Klinikum der Stadt Mannheim
Dept of Dermatology
Postfach 100023
D - 6800 MANNHEIM

Dr. KAGEDAL Bertil
University Hospital
Dept of Clinical Chemistry
S - 581 85 LINKOPING

Dr. KEMALI M.
Ist Cibernetica del CNR
Via Tolana 6
I - 80072 ARCO FELICE/NAPOLI

Dr. KINLEY Judith
Institute for Cancer Research
The Norwegian Radium Hospital
Montebello 0210
Oslo 3
NORWAY

Dr. KLAUS S.
Hadassah Hospital
Dept of Dermatology
PO Box 12000
91-120 Jerusalem
ISRAEL

Dr. KOKOSCHKA E.
University of Vienna
II. Dermatology Dept
Alserstrasse 4
A - 1090 WIEN

Dr. KOLLIAS N.
Kuwait University
Dept of Physics
Safat 133060
KUWAIT

Prof. LAGERLOF B.
Karolinska Hospital
Dept of Pathology
S - 104 01 STOCKHOLM

Dr. LAND E.J.
Paterson Inst. Cancer Res.
Christie Hosp. & Holt Radium
Wilmslow Road
UK - MANCHESTER M20 9BX

Dr. LANG G.
l'Oreal
Av. Eugene Schueller 1
F - 93600 AULNAY SOUS BOIS

Dr. LARSSON Bengt
Uppsala University
Dept of Toxicology
Box 594
S - 751 24 UPPSALA

Dr. LEE C.M.
Unilever Research
Dept of Personal Products
Colworth House
Sharnbrook
UK - BEDFORD MK44 1LQ

Prof. LEJEUNE Ferdy J.
Institut Jules Bordet
L.O.C.B.
Rue Heger-Bordet 1
B - 1000 BRUSSELS

Dr. LENNER L.
Regionsjukhuset
Dept of Clinical Chemistry
S - 58 185 LINKOPING

Prof. LERCH K.
Biochemisches Institut
University of Zurich
Winterthurerstrasse 190
CH - 8057 ZURICH

Dr. LINDQUIST N.G.
University of Uppsala
Dept of Toxicology
Box 594
S - 751 22 UPPSALA

Dr. LINK E.M.
Dept of Chemical Pathology
UCM School of Medicine
Windeyer Building
Cleveland Street
UK - LONDON W1P 6DB

Dr. LOFBERG J.
Uppsala University
Dept of Zoology
Box 561
S - 751 22 UPPSALA

Dr. LOZANO J.A.
Ist. Biochimica Clinica
Conjunto Residencial Apart 61
Murcia
SPAIN

Dr. MAIDA I.
Istituto Biologia Generale
Faculta di Medicina
Piazza Giulio Cesare
I - 70124 BARI

Dr. MALLARDI A.
Istituto Biologia Generale
Faculta di Medicina
Piazza Giulio Cesare
I - 70124 BARI

Dr. MANSSON-BRAHME E.
Radiumhemmet
Karolinska Hospital
S - 104 01 STOCKHOLM

Prof. MARSDEN C.D.
Institute of Psychiatry
Dept of Neurology
De Crispigni Park
UK - LONDON SE5

Prof. MASCARO J.M.
Hosp. Clinico y Provincial
Dept of Dermatology
c/o Casanova 143
08036 Barcelona
SPAIN

Dr. MATOUS B.
Faculty of General Medicine
Dept of Medical Chemistry
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr. MENKE H.E.
St Franciscus Gasthuis
Kleiweg 500
NL - 3045 PM ROTTERDAM

Dr. MEYER zum GOTTESBERGE A.
University of Dusseldorf
Dept of Otorhynology
Moorenstrasse 5
D - 4000 DUSSELDORF

Prof. MEYSKENS F.
Irvine Medical Center
University of California
City Drive South Orange 101
California, CA 92668-3298
UNITED STATES OF AMERICA

Dr. MIRANDA M.
University of l'Aquila
Dept Biol. & Cell Physiology
Via Assergi 6
I - 67100 L'AQUILA

Prof. MISHIMA Yukata
Kobe University Medical School
Dept of Dermatology
5-1, Kusunoki-cho - 7 chome chuo-ku
650 Kobe
JAPAN

Prof. MISURACA G.
University of Naples
Dept of Pharmacology
Via Montesano
I - 80100 NAPLES

Dr. MONFRECOLA G.
Universita di Napoli
Clinica Dermatologica II
Via Pansini 5
I - 80131 NAPOLI

Prof. MacKIE Rona
Glasgow University
Dept of Dermatology
Anderson College Building
56 Dumbarton Road
UK - GLASGOW G11 6NU

Dr. NAPOLITANO A.
University of Naples
Dept Organic & Biol Chemistry
Via Mezzocannone 16
I - 80134 NAPOLI

Dr. NAZZARO-PORRO M.
Instituto Dermatologica
Via San Gallicano 25/A
I - 00153 ROME

Prof. NICOLAUS R.A.
Dept of Chemistry
University of Naples
Via Mezzocannone 16
I - 80134 NAPLES

Dr. NIELSEN H.I.
Medi-Cult a/s
Kanalholmen 12
DK - 2650 HVIDOVRE

Dr. O'HARE K.D.
Keele University
Dept of Biological Science
UK - STAFFORDSHIRE ST5 5BG

Dr. OCHOTORENA M.
University of Murcia
Dept of Pathology
Espinardo
30100 Murcia
SPAIN

Dr. OLANDER-NISS K.
Uppsala University
Dept of Toxicology
Biomedicum
Box 594
S - 75 124 UPPSALA

Dr. ONSMAN I.
Emmakade 52
NL - 1102 AR AMSTELVEEN

Dr. ORECCHIA Giovanni
c/o Clinica Dermatologica/O.S.M.
I - 27100 PAVIA

Prof. ORFANOS C.E.
Univ. Med. Centre Steglitz
Dept of Dermatology
Hindenburgdamm 30
D - 1000 BERLIN 45

Prof. ORTONNE J-P.
Hopital Pasteur
Dept of Dermatology
BP 69
Avenue de la Voie Romaine
F - 06100 NICE CEDEX

Dr. PALUMBO A.
Stazione Zoologica
Villa Comunale
I - 80121 NAPLES

Dr. PARSONS P.
Queensland Inst. for Med. Res.
Bramston Terrace
Herston, Brisbane
QLD 4006
AUSTRALIA

Dr. PASSI S.
Istituto Dermatologica
Via San Gallicano 25/A
I - 00153 ROME

Dr. PAVEL Stan
Akademisch Medisch Centrum
Dept of Dermatology
University of Amsterdam
Meibergdreef 9
NL - 1105 AZ AMSTERDAM

Dr. PETER R.U.
Institute of Radiobiology
Neuherbergstrasse 11
D - 8000 MUNCHEN 45

Prof. PETRES J.
Hautklinik
Dept of Dermatology
Monchebergstrasse 41
D - 3500 KASSEL

Dr. PETTERSON A.
University Hospital
Dept Clinical Chemistry
S - 581 85 LINKOPING

Dr. PICARDO M.
Istituto Dermatologica
Via San Gallicano 25/A
I - 00153 ROMA

Dr. PINTUCCI G.
Ist. Biologia Generale
Policlinico
Piazza G. Cesare
I - 70124 BARI

Dr. POMA A.
University of l'Aquila
Dept Cell Biol & PHysiology
Via Assergi 6
I - 67100 NAPLES

Prof. PROTA Giuseppe
University di Napoli
Dipt Chimica Organica
Via Mezzocannone 16
I - 80134 NAPLES

Dr. PRUNIERAS Michel
l'Oreal
Av. Eugene Schueller 1/22
F - 93600 AULNAY SOUS BOIS

Dr. RAGNELLI A.
University de l'Aquila
Dipto Biol/Fisiologica Cell.
Via Assergi 6
I - 67100 l'AQUILA

Dr. RAMAIAH A.
Dept of Biochemistry
All India Inst. of Med. Sciences
Ansari Nagar
New Dehli 110029
INDIA

Dr. RANSON M.
33 Fernsdale Street
Newton 2042
AUSTRALIA

Dr. RASTRELLI A.
FIDIA s.p.a.
Via Ponte della Fabbrica 3/A
I - 35031 ABANO TERME (PD)

Dr. RENIERI C.
Ist. Produzioni Animali
Via S. Costanzo 4
I - 06100 PERUGIA

Prof. RILEY Patrick A.
University College MSM
Dept of Chemical Pathology
Windeyer Building
Cleveland Street
UK - LONDON W1P 6DB

Dr. RINDONE B.
University of Milano
Dept Organic & Ind. Chemistry
Via Venezian 1
I - 20133 MILANO

Prof. RINGBORG Ulrik
Karolinska Hospital
General Dept of Oncology
S - 104 01 STOCKHOLM

Dr. ROBERTO A.
Uppsala University
Dept of Toxicology
Box 594
S - 571 24 UPPSALA

Prof. RORSMAN Hans
University of Lund
Dept of Dermatology
Lasarettet
S - 221 85 LUND

Dr. ROSDAHL I.
Hudpolikliniken
Lundby sjukhus
Wieselgrensplatsen 19
S - 417 17 GOTEBORG

Prof. SALOMON Y.
The Weizmann Institute of Science
Dept of Hormone Research
PO Box 26
Rehovot 76100
ISRAEL

Dr. SANTORO C.
Istituto Biologia Generale
Via Androne 81
I - 95124 CATANIA

Dr. SARNA T.
Jagiellonian University
Inst. of Molecular Biology
Al Mickiewicza 3
31 120 Krakow
POLAND

Dr. SCALIA M.
Istituto Biologia Generale
Via Androne 81
I - 95124 CATANIA

Prof. SCHACHTSCHABEL D.
Inst. PHysiologische Chemie
Philipps Universitat Lahnberge
D - 3550 MARBURG

Dr. SCHALLREUTER K.U.
University of Hamburg
Dept of Dermatology
Martinistrasse 52
D - 2000 HAMBURG 20

Dr. SCHARTL M.
Max-Planck-Inst. Biochemie
Am Klopferspitz 18
D - 8033 MARTINSRIED

Dr. SCHOTHORST A.
University Hospital
Dept of Dermatology
Bldg 26
PO Box 9600
NL - 2300 RC LEIDEN

Dr. SCHRIER Peter
University Hospital
Dept Clinical Oncology
Bldg I, K1-P
PO Box 9600
NL - 2300 RC LEIDEN

Dr. SCHROTT A.
Dept of ENT
Anichstrasse 35
A - 6020 INNSBRUCK

Prof. SCHUTZ Gunter
German Cancer Research Center
Inst. Cell & Tumor Biology
Im Neuenheimer Feld 280
D - 6900 HEIDELBERG 1

Prof. SERRI Ferdinando
Foundation for Res. in Dermatology
Catholic University
Largo A. Gemelli 8
I - 00168 ROME

Prof. SICHEL G.
Istituto Biologia Generale
Via Androne 81
I - 95124 CATANIA

Dr. SIDDIQUI A.H.
Academic Medical Center
Dept of Dermatology
Meibergdreef 9
NL - 1105 AZ AMSTERDAM ZUIDOOST

Dr. SMIT N.
Academisch Medisch Centrum
Dept of Dermatology
Meibergdreef 9
NL - 1105 AZ AMSTERDAM

Dr. SMOLLE J.
University of Graz
Dept of Dermatology
Auenbruggerplatz 8
A - 8036 GRAZ

Dr. SOLANO-MUNOZ F.
University of Murcia
Espinardo
30100 Murcia
SPAIN

Dr. STIERNER U.
Alfhemsgatan 7
S - 413 10 GOTHENBURG

Dr. TALLBERG T.
Central Hospital
Laboratory of Immunology
Mannerheiminue 172
00280 Helsinki 28
FINLAND

Ms TAYLOR A.J.
Sir William Dunn School Pathol
University of Oxford
South Park Road
UK - OXFORD OX1 3RE

Dr. THODY Anthony J.
Royal Victoria Infirmary
Dept of Dermatology
Univ. Newcastle upon Tyne
UK-NEWCASTLE UPON TYNE NE1 4LP

Mr. THOME D.
Muehlstrasse 31
D - 6300 GIESSEN

Dr. TOSTI Antonio
University of Palermo
Inst. Dermatology & Syphilology
Via del Vespro 131
I - 90127 PALERMO

Dr. URQUHART A.J.
Central Res. & Support Establish.
Home Office Forensic Science Serv.
Aldermaston
Near Reading
UK - READING RG7 4PN

Dr. VERCAMMEN-GRANDJEAN Alain
Institut Jules Bordet
L.O.C.E.
Rue Heger-Bordet 1
B - 1000 BRUSSELS

Dr. VETTERLEIN M.
Inst. Tumor Biology & Cancer Res.
University of Vienna
Borschkegasse 8A
A - 1090 VIENNA

Dr. VICENTE O.
University of Murcia
Dept of Pathology
Espinardo
30100 Murcia
SPAIN

Prof. WEATHERHEAD B.
Dept of Anatomy
Faculty of Medicine
Li Shi Fan Building
5 Sassoon Road
HONG KONG

Prof. WEINREB A.
Racah Institute of Physics
Hebrew University of Jerusalem
91904 Jerusalem
ISRAEL

Dr. WESTERHOF W.
Academic Medical Center
Dept of Dermatology (F7Z)
Meibergdreef 9
NL - 1105 AZ AMSTERDAM

Dr. WILLIAMS I.D.
Pennsylvania State University
Rm 106 Materials Research Lab.
University Park
Pennsylvania, PA 16802
UNITED STATES OF AMERICA

Prof. WOLFF K.
University of Vienna
Dept of Dermatology I
Alserstrasse 4
A - 1090 VIENNA

Dr. WOLFRAM L.J.
Gelb Research Foundation
c/o Clairol Inc.
2 Blachley Road
Stamford, CO 06902
UNITED STATES OF AMERICA

Prof. WYLER H.
Institut de Chimie Organique
Universite de Lausanne
Rue de la Barre 2
CH - 1005 LAUSANNE

Dr. YAMADA K.
Memanbetsu-cho
Abashiri-gun
Hokkaido
JAPAN

Dr. YOUNG Anthony R.
St Thomas's Hospital
Photobiology Unit
Institute of Dermatology
UK - LONDON SE1 7EH

Dr. ZUASTI E.A.
Faculty of Medicine
Dept of Cell Biology
University of Murcia
30100 Murcia
SPAIN

Dr. d'ISCHIA M.
University of Naples
Dept Organic & Biol. Chemistry
Via Mezzocannone 16
I - 80134 NAPLES

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