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LETTER TO THE EDITOR
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Discussion

Post-tyrosinase regulators of melanin synthesis ten years after

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The first indication of post-tyrosinase regulators of melanin synthesis came from a study of the inhibition of melanogenesis by melatonin in the hair follicle of siberian hamsters (1). However, it was not until 1980 that the possible nature and activity of such factors was postulated in a classical paper by Dr. John Pawelek's group (2), when three "distal factors" were postulated :

- a dopachrome conversion factor (DCF), that was thought to accelerate the conversion of dopachrome to 5,6-dihydroxyindole (DHI);
- a 5,6-DHI conversion factor expressed by cells stimulated by MSH and catalyzing the conversion of DHI to melanin;
- a 5,6-DHI blocking factor restricting melanogenesis at 5,6-DHI.

After ten years of active investigation by several teams the most important of these three factors appears to be DCF, since a) dopachrome conversion is a critical step in the biosynthesis of melanins, as noted by Dr. Prota and coworkers (3); b) 5,6-DHI conversion activity can be accounted for by tyrosinase (4); and therefore a new factor does not need to be involved to explain the increased rate of DHI evolution in cells stimulated by MSH; c) as far as we know, no clear cut evidence of the existence of a DHI blocking activity has ever been put forward. This activity has been always measured in crude extracts and no partial purification and characterization have been published. Moreover, its mechanism of action appears difficult to postulate on chemical backgrounds and, as first noted by Dr. King's group at Minnesota, its postulated activity might be accounted for by DCF (5). We will therefore restrict our discussion to the recent findings on dopachrome conversion activity.

There is a rather general agreement as to the nature of the reaction catalyzed by dopachrome. The factor accelerates the conversion of dopachrome into the colorless compound 5,6-DHICA, as first suggested by Korner & Pawelek (6) and latter on unambiguously demonstrated by a variety of techniques including nuclear magnetic

resonance and mass spectrometry (7), HPLC (8,9), and spectrophotometric and radiometric criteria (10,11). Since the transformation of dopachrome into DHICA is a tautomerization, the name dopachrome tautomerase (E.C.5.3.2.3) appears as the most precise and informative and should be preferred to other possible denominations (11). Although there are no doubts about the fact that the spontaneous evolution at neutral pH of the rather unstable compound dopachrome leads to DHI (12), while the action of dopachrome tautomerase leads to the non-decarboxylative rearrangement to DHICA, this very fact has prompted some doubts and controversy as to the actual nature of the catalytic factor. It has been clearly shown that several metal ions, including Cu(II), Co(II) and mostly Ni(II), catalyzed the non-decarboxylative rearrangement of dopachrome to DHICA (3,8,13), and the contents of some of these ions is high in melanoma tissues and probably in the melanosomes of normal melanocytes. Moreover, the total carboxylic group contents of melanins is higher when the pigment is synthesized in the presence of metal ions (14), and these melanins resemble more closely the natural pigment, whose carboxylic group contents is also high (14,15). It has therefore been postulated that the actual regulators of dopachrome rearrangement within the melanocytes could be metal ions, where natural and relative concentrations might account for some of the differences in natural melanin obtained from various sources.

Should we therefore come back to the first idea that tyrosinase is the only enzymatic factor controlling melanosynthesis from tyrosine ? We think that, although the catalytic effect of metal ions on dopachrome rearrangement are indisputable (3,8,13), their actual importance in vivo is not clear. On the other hand, the evidence pointing to the existence of a specific protein, dopachrome tautomerase, within the melanocyte is overwhelming, and even be summarized as follows :

- the activity has been partially purified by a variety of chromatographic techniques to high specific activity (5,9,11). Dr. King's group has reported the purification of the enzyme to electrophoretic homogeneity (XIVth International Pigment Cell Conference, Kobe). The finding of active and inactive chromatographic fractions i.e. of a specific chromatographic profile excludes the possibility that the activity might be explained by a contamination of buffers by metal ions;

- the activity is heat-sensitive and protease-sensitive as shown by a variety of authors;

- the activity of purified preparations is not affected by the inclusion in the assay media of high concentrations of EDTA (11) or by Chelex 100 treatment of buffers (8);

- dopachrome tautomerase is competitively inhibited by some analogues of the substrate and product including L-Trp (11) and DHICA itself (16);

- purified dopachrome tautomerase is highly stereospecific and does not catalyze the decarboxylative rearrangement of D-dopachrome (16);

- the hydrolytic action of a variety of glycosidases activates the dopachrome tautomerase activity (results to be published). All these observations leave little if any doubt as to the actual existence of a specific enzyme, specially if one considers that dopachrome tautomerase activity is high, and, in B16 melanoma melanocytes, comparable in terms

of units/g of tissue to tyrosinase activity. In light of the available evidence, two matters appear therefore settled : the existence of a distinct protein catalyzing dopachrome conversion, and the nature of its reaction product, DHICA, which immediately sets the name dopachrome tautomerase as the most appropriate for the protein. However, several points await further clarification and might be the subject of future research. We will just mention three of them.

1. As we mentioned above, both metal ions and dopachrome tautomerase are able to catalyze the same reaction, and the concentration of both types of reagents appear to be high in the melanosomes. It will be therefore important to determine which one of the two is the actual physiological regulator of dopachrome evolution, although their action is not mutually exclusive and might even be synergistic (13). Research in this field should undoubtly be speeded up by the collaboration between the different teams involved and by the standardization of the assay methods for metal ions and dopachrome tautomerase activity.

2. The relationship between dopachrome tautomerase and indole blocking factor first noted by the Minnesota group (5) is also interesting. In the presence of dopachrome tautomerase (or metal ions) the evolution of dopachrome leads to polymeric products more soluble and less dark than the ones obtained spontaneously (5,11°, and similar to DHICA-melanins (15). Visually, dopachrome tautomerase behaves as an indole blocking factor, although we have shown that the enzyme actually increases the rate of formation of a melanin-like polymer, in systems containing either tyrosine or dopachrome and tyrosinase and catalytic amounts of dopa (11,13, results to be published). It is therefore possible that the postulated indole blocking activity might be an artifact of visual or spectrophotometric estimates of melanin formation rates, and might be accounted for by the formation of DHICA-melanins, rather than DHI-melanins in the presence of the enzyme. A definitive answer might come from a more systematic search of the blocking activity and its comparison to the tautomerase activity.

3. In addition, a protein factor that shows strong similarities to the specificity of dopachrome tautomerase has been described in insects (17). This factor has been named dopaquinone imine conversion factor. Therefore, it is plausible that enzymes catalyzing the tautomerization of dopachrome could occur in different organisms of the phylogenetic scale. The widespread existence of such enzyme would support the view that it might play an important role in the regulation of melanin biosynthesis.

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Short communication

Tumor associated genes in Xiphophorus - Inheritance and expression of x-erbB*, x-erbB, x-pdgf, x-pdgf-r, and x-erbA

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Certain genotypes of *Xiphophorus* harbour a Mendelian tumor gene complex (Tu-complex) which mediates the hereditary capacity to develop neoplasia spontaneously or following induction with initiating and promoting carcinogens. We concentrated on sex chromosome-linked tumor formation.

We cloned and partially sequenced xiphophorine genes homologous to the human c-erbB-1 (h-egfr), c-sis (h-pdgf-2), PDGF-receptor gene (h-pdgf-r) and c-erbA (h-erbA) and studied the inheritance of thse genes in animals with genetic predisposition to tumor formation. So far, only a certain type of x-erbB (x-erbB*) appears to be inherited in parallel with the Tu-complex. This x-erbB*, disclosed by an X-chromosomal 4.9 kb or another 11.5 kb, and an Y-chromosomal 6.7 kb Eco R1 fragment, map to the locus of the melanoma determining Tu-complex; these genes, x-erbB*a, which are accessory show high homology to an indispensable x-erbB*i. Expression of the x-erbB*i was detected in testes, brain, gills and during embryogenesis. The x-erbB*i is expressed atto a low level in melanoma, fibrosarcoma, neuroblastoma and thyroid tumors. Fish that carry the sex-chromosomal x-erbB*a express or overexpress x-erbB* in melanoma, certain fibrosarcoma and thyroid tumors. Furthermore, all tumors studied express x-pdgf-r and most of them express x-pdgf. A second type of x-erbB that was disclosed by an autosomal 5.5 kb band and probably represents the common egfr shows expression in melanoma and fibrosarcoma. Genes related to h-erbA that show differential expression during embryogenesis are expressed in normal tissue of young and adult fish but show low or no expression in tumors. So far, only the x-erbB* appears to be involved in the initial steps leading to melanoma and possibly to certain other tumors. The role of pdgf, pdgf-r, x-erbB, and x-erbA in tumor formation remains unclear.

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Short communication

erbB*a, an "ignition system" of the xiphophorine melanoma machinery ?

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EXPERIMENTAL FISH. Certain platyfish/swordtail hybrids are susceptible to neoplasia: some are sensitive to carcinogens, others to tumor promoters, while others develop neoplasia "spontaneously". Susceptibility to melanoma depends on an incompletely derepressed, terminally located sex chromosomal or autosomal Mendelian gene complex (Tu) composed of a pterinophore locus, 14 compartment-specific genes suppressing melanoma, a v-erbB related gene and a melanoblast determining gene (x..,R^{Col-14}, erbB*a, Mel), all of which are accessory. The melanomas studied were from BC_n hybrids carrying 1 platyfish Tu in a platyfish or swordtail chromosome (interspecific translocation), and 47 swordtail chromosomes lacking Tu. According to Southern blot, the "transgene-like" accessory x-erbB*a is the only platyfish-derived oncogene in the host. When x-erbB*a was deleted, no melanomas developed.

RESULTS. x-erbB*a is always overexpressed in the melanomas. However, melanomas also show expression of the indispensable x-erbB*i, and variable expression of x-src, x-sis, x-pdgf-r, x-ras, x-myc, x-myb and x-erbA. These genes are swordtail-derived but are apparently driven by the platyfish x-erbB*a. Similarly, the melanomas show a 10-fold elevation of the oncogene-interrelated phosphatidylinositol (PtdIns) turnover which, for genetic reasons, must be contributed by the swordtail genome. In hybrids developing hereditary melanoma, x-src activity and PtdIns turnover is also elevated in the brain. Introduction of a gene that retards differentiation of stem melanoblasts prevents melanoma, but does not diminish the high x-src activity and PtdIns turnover in the brain. The outgrowth of melanoma is decoupled from its preceeding biochemical processes driven by x-erbB*a. Recouplement of these processes by cell differentiation stimulated by tumor promoters "induces" the melanoma outgrowht.

CONCLUSIONS. Many components of the melanoma machinery are apparently driven by x-erbB*a, just as engines are controlled by an ignition system, i.e. the initial ignition-spark and the maintenance of an ignition-timing.

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Review

Pigment Cell Research in Australasia

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The aim of this review is to briefly outline current research areas. A few key references have been included for further information. Detailed accounts of the epidemiology and treatment of melanoma in Australia and New Zealand have recently been published (Trans. Menzies Foundation, vol. 15, 1990; Cancer Forum, 14:1-34, 1990; Med. J. Aust. 150: 469-470, 1989).

Pigment cell research in Australia is centred largely on the biology and treatment of human melanoma, due to the 1 in 50 lifetime risk of this tumour. Thus the state of Queensland, one of the few subtropical areas in the world sustaining a large Caucasian population, has the highest incidence globally (40 per 10^5 per annum) and apparently doubling each decade. Since Lancaster's original suggestion of a solar aetiology (Med. J. Aust., 1:452, 1957) there have been many epidemiological studies of melanoma in Australia, notably Armstrong's group in Perth documenting the risk associated with exposure in childhood (JNCI, 72:257-266, 1984) and Green and others in Brisbane describing moles and sunburn as major risk factors in adults and children (Epidemiol. Rev., 11:204-221, 1989; J. Am. Acad. Dermatol 20:1054-1060, 1989). Following on from earlier work (Ann. Human Biol., 8:529-541, 1981), Martin and co-workers in Brisbane are studying naevus patterns and skin relectance in twins with and without melanoma. McLennan et al. are looking at the dependence of naevi formation on latitude and other factors. Across the Tasman Sea in the scenic South Island of New Zealand, Elwood and his colleagues at the University of Otago in Dunedin are involved in the epidemiology of naevi and melanoma (Int. J. Cancer, 36:175-178, 1985; N.Z. Med. J., 101:602-604, 1988). There is a perceived need to compare the epidemiology and biology of nonmelanoma skin cancers (associated with chronic solar exposure) with that of melanoma (associated with acute exposure) so that a biological basis for the latter can be established, and there is concern about the ozone layer particularly the Antarctic ozone hole. The incident UV flux and exposure times in Australia are probably much higher than in the northern hemisphere and consequently may give different patterns of skin carcinogenesis.

Despite the large population of cattle and sheep in Australia melanoma appears to be rare in these animals. Recently a significant number of melanomas has been found in goats, enabling a melanoma cell line to be established (Parsons et al, in press). Reeve and Greenoak in Sydney use hairless, pigmented mice for a variety of studies including evaluation of sunscreens and antioxidants (Photochem. Photobiol. 48:689-696, 1988).

Despite the high risk of melanoma, the death rate (about 4 per 10^5 per annum) remains relatively low, presumably reflecting the success of early and effective surgery in a population alerted to the problem. Supported by government funds and state cancer

societies, imaginative education programmes aimed particularly at young people urge protection from solar UV by all possible means and early removal of suspicious lesions. A television documentary featuring a young man with melanoma directly resulted in approximately 750 new cases of melanoma being diagnosed in the following 6 months (McCarthy and Shaw, Cancer Forum 14:6-10, 1990).

Financed mainly by the state cancer societies and the federal National Health & Medical Research Council, research on melanoma in Australia owes much to the logistic support of the clinicians who established and maintain centres for treatment and research. These groups include the Sydney Melanoma Unit, which is the largest melanoma treatment centre in the world, and the Queensland Melanoma Project in Brisbane. In addition to making important contributions to the diagnosis and treatment of melanoma, these centres identify and monitor melanoma-prone families and collaborate in linkage studies of familial melanoma/dysplastic naevus syndrome (Kefford et al, Cancer Genet. Cytogenet., in press) and mapping melanoma-associated genes or chromosomal regions (Dracopoli et al, PNAS, 86: 4614-4618, 1989; Hayward et al, Int J. Cancer, 42:558-561, Human Genet. 83: 395-396, 1989). Unlike certain families in the United States, Australian melanoma families have so far shown no evidence of linkage to chromosome 1p. Such differences may be part of the heterogeneity and genetic instability which bedevils attempts to understand and control melanoma.

The establishment of human melanoma cell lines by Whitehead and Little at the Queensland Institute of Medical Research in Brisbane (Pigm. Cell 1:383-389, 1973) marked the beginning of a wide range of experimental studies of which only some current aspects can be mentioned here. Evidence that TGFα is a mediator of the UV response in melanocytic cells (Ellem et al, Carcinogenesis, 9:797-801, 1988) has been confirmed in studies of sun-irradiated humans; regulation of TGFα activity is now being examined. Direct sunlight is being used as an experimental light source to induce mutation in melanocytic cells where aberrant nucleoside metabolism may play a role in enhancing mutagenesis (Musk et al, Mutat. Res. 227:25-30, 1989). Other peculiarities of cultured melanoma cells under study include deficiency in DNA methylation repair (Maynard et al, Cancer Res., 46:5009-5013, 1989) and sensitivity to buthionine sulphoximine (Kable et al, Cancer Res., 49:2327-2331, 1989). This work overlaps with analysis of pigmentation control, key tools being in-house monoclonal antibodies, affinity purification and N-terminal sequencing of active sulphydryl proteins, glycosylation inhibitors, novel marine compounds, UV-induced factors, and immunohistochemistry of clinical material (Takahashi et al., Virchows Archiv. A, 416:513-519, 1990). In Sydney, natural modulators of melanogenesis including Vitamin D are under study in melanoma cells (Mason et al, J. Invest. Dermatol., 90:334-340, 1988) and melanocytes (idem, 90:593-598, 1988).

Non-surgical therapy of melanoma is being investigated in several centres. McLeod and Thomson in the Queensland Melanoma project are involved in clinical trials of interferon and DTIC (Br J. Clin. Pract. Symp. Suppl., 62:22-26, 1988) as well as video imaging of dysplastic naevi, quality of life studies and maintenance of an extensive data bank. The Sydney Melanoma Unit has used a variety of agents (Coates et al, Pigm. Cell Res., 2: 370-371, 1989), including neutron capture therapy in collaboration with Allen and his colleagues (Brown et al, Pigm. Cell Res., 2: 319-324, 1989). The Melanoma Unit in Newcastle has a particular interest in immunotherapy, currently involving clinical trials

of immunization with vaccinia melanoma cell lysates (Hersey et al., Cancer Immunol. Immunotherapy 25:257-265, 1987). In vitro analysis of melanoma antigens is based on detection by antibodies using Western blot techniques (Int. J. Cancer, 46: 612-617, 1990) and T cell responses using nitrocellulose immobilized antigen (J. Natl. Canc. Inst. 80:826, 1988). Studies in Newcastle also include assessment of the effects of UV on the human immune system, in particular the dose dependent inhibition of NK activity by UV-A.

Although these groups are geographically distant, researchers meet regularly at national conferences, for example the Clinical Oncology Society of Australia conference in November, as well as at melanoma meetings held from time to time on special topics. Whether for a conference, sabbatical, postgraduate degree or fellowship period, travel to Australia offers a variety of opportunities for pigment cell researchers as well as interesting natural environments and the double-edged attraction of a sunny climate. Australian researchers actively collaborate with overseas groups and welcome enquiries from prospective visitors, who are encouraged to make contact at an early stage so that suitable arrangements can be made.

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CURRENT LITERATURE

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

- **Bathory G, Szuts T, Magyar K.**
Properties of the binding of 1-deprenil (Jumex) to melanin pigment. **Acta Pharm Hung 59 (Suppl 1):69-74, 1989.**

- **Cegarra J, Gacen J, Shumacher-Hamedat U, Knott J, Biankenburg G, Caro M.**
Depigmentation of fine animal hairs. **Bol INTEXTAR Inst Invest Text Coop Ind 96:11-26, 1989.**
Abstract : A depigmentation method for fine alpaca, camel, and yak hair is based on formation of melanin-Fe2+ complexes during mordant treatment with FeSO4 and reducing agents, rinsing for removal of noncomplexed Fe2+, and bleaching with H2O2. Removal of Fe2+ is crit. to prevent damage to the fibers and is effectively carried out using EDTA and Na nitrilotriacetate (I) during washing. For all samples, I was more effective than EDTA in terms of depigmentation and fiber quality; also, I is more biodegradable than EDTA. During bleaching, the complexed pigments are selectively destroyed at a fast rate and fibers with very little or no coloration are obtained.

- **Czapla TH, Hopkins TL, Kramer KJ.**
Catecholamines and related o-diphenols in cockroach hemolymph and cuticle during sclerotization and melanization: comparative studies on the order Dictyoptera. **J Comp Physiol [B] 160:175-181, 1990.**
Abstract : Catecholamines and related o-diphenols extracted from the cuticle and hemolymph of adult cockroaches during sclerotization and pigmentation of the cuticle were analyzed by reverse phase HPLC with electrochemical detection. At ecdysis, dopamine (DA) o-conjugates predominated in the hemolymph of Periplaneta americana, P. australasiae, P. fuliginosa, P. brunnea, and Blatta orientalis (Blattidae); Blattella germanica (Blattellidae); and Gromphadorhina portentosa and Blaberus craniifer (Blaberidae). N-Acetyldopamine (NADA) conjugates were second in abundance in these species, but were major in the hemolymph of the other blaberoid species, Leucophaea maderae and Nauphoeta cinerea. After ecdysis NADA became the major hemolymph catecholamine in all species as DA decreased rapidly. N-beta-Alanyldopamine (NBAD) concentrations in the hemolymph remained low in all species, although NBAD and its metabolite, N-beta-alanylnorepinephrine (NBANE), were generally the major catecholamines in tanning cuticle. Catechol (1,2-dihydroxybenzene) occurred mainly as a conjugate(s) at high levels in the hemolymph of nymphs and adults of all blattid species. Only trace amounts were detected in B. germanica and Cryptocercus punctulatus (Cryptocercidae), and none was found in any of the blaberoid species. High concentrations of NBANE and NBAD accumulated in tanning cuticle of B. germanica, G. portentosa, and all blattid species, whereas NADA and DA predominated in cuticle from the other blaberoid species, particularly L. maderae and N. cinerea. However, cockroaches as a group appear to utilize both the N-acetyl and N-beta-alanyl catecholamines for stabilization of the exoskeleton. The Blattidae differed most from the other families in having considerably higher concentrations of catecholamines in hemolymph and cuticle, as well as the large amounts of catechol conjugates in the hemolymph.

- **Gacen Guillen J, Cegarra Sanchez J, Caro Silanes M, Pepio Vinals M.**
Bleaching with hydrogen peroxide of selectively prebleached wool. **Bol INTEXTAR Inst Invest Text Coop Ind**

96:49-60, 1989.

Abstract : The whiteness of wool bleached with H2O2 after selective mordant pretreatment with FeSO4 shows little improvement over wool treated only with H2O2; the fibers from pretreatment are less white and present more chem. damage than untreated fibers. The pretreatment is based on formation of complexes of Fe2+ and melanin, followed by selective destruction of complexed pigment during bleaching. For pretreated and not treated wool, the optimum temp. of the bleaching bath was 50 and 45.degree., resp., and the time 3-4 h.

- **Ingebrigtsen K, Skoglund LA, Nafstad I.**

A study on melanin affinity of 14C-trimethoprim in male Mol:WIST and Mol:PVG rats. **Z Versuchstierkd 33:73-77, 1990.**

Abstract : Hooded (Mol:PVG) and albino (Mol:WIST) rats were used to study the distribution pattern of 14C-trimethoprim by whole body autoradiography. Accumulation of radioactivity was demonstrated in the uveal tract of the eye and the pigmented parts of the skin in the hooded rats. No radioactivity was present in the corresponding tissues in the albino rats. This difference in the distribution pattern was interpreted to reflect binding to melanin of 14C-trimethoprim and/or its metabolites.

- **Korytowski W, Sarna T.**

Bleaching of melanin pigments. Role of copper ions and hydrogen peroxide in autooxidation and photooxidation of synthetic dopa-melanin. **J Biol Chem 265:12410-12416, 1990.**

Abstract : Bleaching of eumelanin has been studied in model systems consisting of synthetic dopa-melanin and various concentrations of hydrogen peroxide, molecular oxygen, and copper(II) ions at neutral and alkaline pH. The data show that at neutral pH, in the dark, metal-ion-free melanin is very resistant to oxidation by hydrogen peroxide. However, the rate of bleaching of melanin, induced by H2O2 is significantly accelerated by illumination from UVA-visible light. Bound-to-melanin copper(II) also accelerates the bleaching of melanin with the efficiency dependent on concentration of H2O2 and oxygen. It suggests possible involvement of melanin-copper complexes in Fenton-like processes. The formation of hydroxyl radicals during melanin bleaching has been concluded on the basis of the electrochemical detection of hydroxylation products of salicylate used as OH scavenger. Redox conversion of bound-to-melanin copper ions was monitored by EPR spectroscopy and direct measurement of melanin-Cu(II) complexes. It has been shown that melanin-copper(I) complexes were readily oxidized by either oxygen or hydrogen peroxide. The data indicate that bleaching of melanin is a complex process with two distinct stages, reversible oxidation of the hydroquinone moieties of melanin followed by irreversible reactions of the monomers that lead to degradation of the melanin polymer.

- **Menter JM, Townsel ME, Moore CL, Williamson GD, Soteres BJ, Fisher MS, Willis I.**

Melanin accelerates the tyrosinase-catalyzed oxygenation of p-hydroxyanisole (MMEH). **Pigment Cell Res 3:90-97, 1990.**

Abstract : Although pigment melanin has long been though of as "inert," recent work has attested to its chemical reactivity. In this communication, we report that either commercial synthetic melanin prepared by persulfate oxidation of tyrosine ("Sigma melanin") or sepia melanin extracted from cuttlefish markedly accelerates the in vitro oxygenation of p-hydroxyanisole (MMEH), catalyzed by mushroom or B-16 melanoma tyrosinase. Kinetics of 4-methoxy-1,2-benzoquinone formation (lambda max = 413 nm) or of molecular O2 uptake were biphasic, with an initial slow rate ("lag time") followed by a fast linear increase. The biphasic response reflects an initial slow hydroxylation followed by a fast dehydrogenation. Added melanin markedly decreased the lag time but had little effect on subsequent dehydrogenation. Similar effects were observed for tyrosine itself. A complex between MMEH and melanin appears to be the "active" species in these reactions. The results indicate that melanin acts as an electron conduit, which accepts electrons from the substrate and transfers them to tyrosinase. The magnitude of the effect depends on the type of melanin as well as on its oxidation state. Kinetic analysis indicates that both melanins are very efficient at transferring electron to tyrosinase, and that Sigma melanin is roughly threefold more efficient than sepia melanin. The qualitative similarity of reaction between the synthetic and "natural" melanins suggests that the former may serve as a first approximation to the in vivo situation. On the other hand, the observed quantitative differences and the sensitivity of these results to the chemical state of melanin suggests that this methodology migh eventually be adapted as a non-destructive probe of melanin in situ.

- **Monks TJ, Highet RJ, Lau SS.**
Oxidative cyclization, 1,4-benzothiazine formation and dimerization of 2-bromo-3-(glutathion-S-yl)hydroquinone. **Mol Pharmacol 38:121-127, 1990.**
Abstract : Several lines of evidence suggest that the renal-specific toxicity of quinol-linked GSH conjugates is probably a result of their metabolism by gamma-glutamyl transpeptidase and selective accumulation by proximal tubular cells. Transport of the resultant quinol-cysteine and/or cystein-S-ylglycine conjugate followed by oxidation to the quinone may be important steps in the mechanism of toxicity of these compounds. Factors modulating the intracellular and/or intralumenal concentration of the cystein-S-yl and cystein-S-ylglycine conjugate will, therefore, be important determinants of toxicity. We have now studied the gamma-glutamyl transpeptidase-mediated metabolism of 2-bromo-3-(glutathion-S-yl)hydroquinone. The product of this reaction, 2-bromo-3-(cystein-S-ylglycyl)hydroquinone, undergoes an intramolecular cyclization to yield a 1,4-benzothiazine derivative that retains the glycine residue. A similar cyclization reaction occurs with 2-bromo-3-(cystein-S-yl)hydroquinone, which is unstable in aqueous solutions and undergoes a pH-dependent rearrangement that requires initial oxidation to the quinone. UV spectroscopy revealed that, at neutral pH, further reaction results in the formation of a chromophore, consistent with 1,4-benzothiazine formation. This product arises via cyclization of the cysteine residue via an intramolecular 1,4 Michael addition. Further reaction results in the precipitation of a pigment that exhibits properties of a pH indicator. The pigment undergoes a marked pH-dependent bathochromic shift (approximately 100 nm); it is red in alkali (lambda max, 480 nm) and violet in acid (lambda max, 578 nm). These properties are similar to those of the trichochrome polymers that are formed during melanin biosynthesis from S-(3,4-dihydroxyphenylalanine)-L-cysteine. Because the intramolecular cyclization reactions remove the reactive quinone moiety from the molecules, they may be regarded as detoxication reactions. 1,4-Benzothiazine formation represents a novel pathway that diverges from the usual route of mercapturic acid synthesis and may represent previously unrecognized and important products of quinone metabolism in vivo.

- **Ohyama Y, Mishima Y.**
Melanogenesis-inhibitory effect of kojic acid and its action mechanism. **Fragrance J 18:53-58, 1990.**
Abstract : A review with 17 refs. on the inhibitory effect of kojic acid isolated from fermentative products of Aspergillus species on melanogenesis through chelation of the metal ions of tyrosinase and a precursor monomer of melanin polymer (5,6-dihydroxyindole 2-carboxylic acid), and its therapeutic effect on UV-induced hyperpigmentation and pigmentary disorders of human skin.

- **Okazaki K.**
Skin-lightening preparations containing unsaturated linear fatty acids. **Jpn Kokai Tokkyo Koho, 6 pp., 1990.**
Abstract : Cosmetic prepns., which are safe and prevent melanin formation, contain C4-17 linear fatty acids having 1-4 unsatd. bonds at any positions except for CO2H-free terminals (10-pentadecenoic acid and 9-hexadecenoic acids are excluded) or their derivs. 9-Tetradecenoic acid 0.05, poly(oxyethylene) stearyl ether 2.0, poly(oxyethylene) cetyl ether 2.0, beeswax 6.0, cetanol 6.0, iso-Pr palmitate 10.0, liq. paraffin 30.0, polyethylene glycol monostearate 1.0, Me p-hydroxybenzoate 0.2, and H2O to 100% were mixed to give a cream.

- **Okazaki K.**
Skin-lightening preparations containing unsaturated linear alcohols. **Jpn Kokai Tokkyo Koho, 4 pp., 1990.**
Abstract : Cosmetic prepns., which are safe and prevent melanin formation, contain C4-17 aliph. linear alcs. having 1-4 unsatd. bonds at any positions except for OH-free terminals or their derivs. 9-Hexadecenol 0.05, poly(oxyethylene) stearyl ether 2.0, poly(oxyethylene) cetyl ether 2.0, beeswax 6.0, cetanol 6.0, iso-Pr palmitate 10.0, liq. paraffin 30.0, polyethylene glycol monostearate 1.0, Me p-hydroxybenzoate 0.2, and H2O to 100% were mixed to give a cream.

- **Okazaki K.**
Comparison of enhanced and routine methods for measuring ambient low-level sulfur dioxide. **Jpn. Kokai Tokkyo Koho, 7 pp., 1990.**
Abstract : Transdermal prepns., which are safe and prevent melanin formation, contain 6-octadecenoic acid (l), 11-octadecenoic acid, 11,14-eicosadienoic acid, 8,11,14-eicosatrienoic acid, 11,14,17-eicosatrienoic acid, 5,8,11,14-eicosatetraenoic acid, 5,8,11,14,17-eicosapentaenoic acid, 6-octadecenol, 11-octadecenol,

11,14-eicosadienol,8,11,14-eicosatrienol,11,14,17-eicosatrienol,5,8,11,14-eicosatetraenol,5,8,11,14,17-eicosapentaenol, and/or their derivs. as active ingredients. I 0.5, poly(oxyethylene) stearyl ether 2.0, poly(oxyethylene) cetyl ether 2.0, beeswax 6.0, cetanol 6.0, iso-Pr palmitate 10.0, liq. paraffin 30.0, polyethylene glycol monostearate 1.0, Me p-hydroxybenzoate 0.2, and H2O to 100% were mixed to give a cream. For the accurate measurement of ambient SO2 concns. <2 ppb, a more sensitive instrument (TECO43A) with a manufacturer reported MDL of 0.6 ppb and a more demanding data redn. and processing scheme produced an enhanced sensitivity of 0.2 ppb. At this sensitivity level, measurements were above the MDL, >80% of the time, which compares to 34% of the time for the moderately sensitive method.

- **Oyanagui Y.**
SOD and melanin formation. **Fragrance J 18:94-97, 1990.**
Abstract : The relation between active O species and melanin formation is described. Active O species (O2-, H2O2, OH.bul., OCl-, 1O2) damage directly the skin and form lipid peroxides which result in insol. pigments. Eumelanins (black, dopamelanin) and pheomelanins (red, dopa/cysteine-melanin) can be formed from tyrosine by UV via prodn. of active O species. Melanins not only quench active O species to protect the body but also increase OH.bul. when Fe and EDTA are present. Quenching active O species is a way to prevent the formation of melanins. Liposomal superoxide dismutase (SOD) in cream is reported to decrease lipid peroxides in the skin after UV irradn. An invention of vehicle to fix antioxidants on skin seems essential for future developments to keep the skin white.

- **Sugumaran M, Dali H, Semensi V.**
Formation of a stable quinone methide during tyrosinase-catalyzed oxidation of .alpha.-methyl dopa methyl ester and its implication in melanin biosynthesis. **Bioorg Chem 18:144-153, 1990.**
Abstract : Mushroom tyrosinase-catalyzed oxidn. of .alpha.-Me dopa Me ester formed iminochrome similar to the well-known dopa-dopachrome conversion. At pH 5, the iminochrome formed was stable and accumulated in the reaction mixt. However, when the reaction was carried out under near neutral and slightly alk. conditions, the iminochrome formed readily tautomerized into a quinone methide isomer, which accumulated in the reaction mixt. The structure of the quinone methide formed was established by UV and visible spectroscopy and cochromatog. with a synthetic sample. These results demonstrate the facile generation of quinone methide from iminochrome under physiol. conditions and strongly support the possible occurrence of a similar reaction during melanogenesis.

- **Tsutsumi T.**
Cosmetics inhibiting melanin formation. **Jpn Kokai Tokkyo Koho, 4 pp., 1989.**
Abstract : A topical cosmetic inhibiting melanin formation contains glabridin (I), glabrene, or a Glycyrrhiza glabra ext. contg. these compds. Thus, a skin-lightening cosmetic consisted of I 0.003, stearic acid 15.0, cetanol 1.0, KOH 0.7, glycerin 5.0, propylene glycol 3.0, a preservative q.s., and H2O 75% by wt. A method of I extn. from the root of G. glabra is shown.

- **Uematsu T, Sato R, Fujimori O, Nakashima M.**
Human scalp hair as evidence of individual dosage history of haloperidol: a possible linkage of haloperidol excretion into hair with hair pigment. **Arch Dermatol Res 282:120-125, 1990.**
Abstract : We report a method for determining haloperidol concentration in human scalp hair and discuss a possible linkage of haloperidol excretion into hair with the hair pigment melanin. First, an animal study was conducted to support the idea that hair contains amounts of haloperidol corresponding to the doses given and pigmented hair contains much more drug than does unpigmented hair. The haloperidol concentration was measured using a radioimmunoassay technique after hairs were dissolved in 2.5 N NaOH solution and the drug extracted. Pigmented and albino rats, whose hair from an area on the back had been removed beforehand by plucking, were administered either 1, 3, or 10 mg of haloperidol (i.p.) per kg body weight every day for 3 weeks. At the end of the administration period hair which had newly grown on the denuded area was plucked and collected. In each of the two groups classified by hair color the drug levels in the hair correlated with the doses given; however, the concentrations in the hair from the albino rats were much lower than those in the hair from the pigmented rats (which was less than 8.5%). Second, black and white hair was collected from each of seven human subjects with grizzled hair, who were receiving or had been administered haloperidol at fixed daily doses for more than 1 month, and the concentration of haloperidol

in each type of hair was measured. In the same subject the concentration in the white hair was found to be much lower than that in the black (< 10%).

- **Wilczok T, Stepien K, Buszman E, Porebska-Budny M.**
Interaction of methotrexate with melanins and melanosomes from B16 melanoma. **Biophys Chem 35:265-270, 1990.**
Abstract : It has been demonstrated that methotrexate forms stable complexes with melanin and melanosomes isolated from B16 melanoma. The number of binding sites and binding constants for methotrexate binding by intact melanosomes and melanin were n = 0.046 mumol/mg, K = 0.32 x 10(4) M-1 and n = 0.063 mumol/mg, K = 1.08 x 10(4) M-1, respectively. Binding of methotrexate to synthetic DOPA-melanin used for comparison also shows a single class of binding sites, n = 0.060 mumol/mg with binding constant K = 2.34 x 10(4) M-1. The possibility of side effects caused by methotrexate-melanin interactions after treatment of neoplasms is discussed.

- **Wolfram L, Schultz TM, Chan AC.**
Skin tanning compositions containing melanin precursors. **Eur Pat Appl, 8 pp., 1990.**
Abstract : A tanning compn. comprises .gtoreq.1 melanin precursors selected from the group consisting of 3,4-dihydroxyphenylalanine, 5,6-dihydroxyindole, N-alkyl-5,6-dihydroxyindole, and derivs. thereof. The tanning compn. is applied to the skin and the skin is exposed to UV radiation to generate color in the stratum corneum. A lotion contained N-methyl-5,6-diacetoxyindole 1, iso-Pr myristate 1.5, EtOH 8, triethanolamine 0.5, Carbopol-940 0.2, and water to 30 g.

- **Zink P, Fengel D.**
Studies on the coloring matter of blue-stain fungi. Part 3. Spectroscopic studies on fungal and synthetic melanins. **Holzforschung 44:163-168, 1990.**
Abstract : Melanins isolated from the blue-stain fungi, Ceratocystis coerulescens and Alternaria alternata, as well as 3 synthetic melanins were studied using various spectrometric methods. The melanins of the 2 fungi were very similar in chem. structure, and they contained a high no. of aliph. groups, whereas the synthetic melanins were predominantly arom. The fungal melanins were assocd. with carbohydrates and proteins which could be removed by acid hydrolysis, but part of the aminic elements remained in the residual melanin structure. The aliph. elements of the fungal melanin mols. were C6-C12 chains which were attached to phenolic rings.

2. Biology of pigment cells and pigmentary disorders

- **Aszakies C.**
Fundamentals of fur coloration in rabbits (Oryctolagus cuniculus). **Wiss Z Wilhelm-Pieck-Univ. Rostock, Naturwiss. Reihe, 37(6):69-74, 1988.**
Abstract : A review, with 62 refs., describing morphol., biochem., and genetic factors which control melanin pigmentation in rabbit fur.

- **Brooks G, Birch M, Hart IR.**
Effects of biologically active tumour-promoting and non-promoting phorbol esters on in vitro growth of melanocytic cells. **Pigment Cell Res 3:98-100, 1990.**
Abstract : Sapintoxin A (SAP A), a naturally occurring biologically active but non-promoting phorbol ester, acts as an effective in vitro mitogen for freshly derived human melanocytes. Seven days after addition of 50 nM SAP A there was a four to fivefold increase in melanocyte number over that observed in untreated control cultures comparable to that achieved with a 50 nM concentration of 12-0-tetradecanoylphorbol 13-acetate (TPA). The fluorescent stage 2 promoter sapintoxin D (SAP D) also supported the growth of these cells, with a 50 nM dose producing an increase in cell number comparable to that observed with 200 nM TPA. Similar results were obtained with an established, but non-tumorigenic, line of murine melanocytes. The same compounds exerted a potent anti-proliferative effect against transformed melanocyte lines of murine and human origin associated with morphological alterations and an increase in melanin production consistent with induced cytodifferentiation.

- **Bundza A, Feltmate TE.**
Melanocytic cutaneous lesions and melanotic regional lymph nodes in slaughter swine. **Can J Vet Res 54:301-304, 1990.**
Abstract : During a five month period, 220 slaughter swine (at two abattoirs) had gross cutaneous and lymph node lesions suggestive of melanoma. Lymph nodes from 214 and cutaneous lesions from 176 of these pigs were submitted for histological examination. Of the cutaneous lesions, 174 were spontaneously regressing melanomas, and two were nonregressing. Regression usually commenced by infiltration of the lesion by lymphocytes, plasma cells and the formation of giant cells. Of the melanotic lymph nodes, 177 were diagnosed as melanosis, 35 were considered to be metastatic regressing melanomas, and two were nonregressing melanomas. This report indicates a high rate of spontaneous regression in swine melanomas detected at slaughter.

- **Chakraborty DP, Roy S, Chakraborty AK, Rakshit R.**
Tryptophan participation in melanogenesis: modification of Raper-Mason-Pawelek scheme of melanin formation. **J Indian Chem Soc 66:699-702, 1989.**
Abstract : The biomimetic synthesis of melanin from tryptophen using the Udenfriend system (Fe2+/ascorbic acid/EDTA/O2) is described. On the basis of these results, a modification of the Raper-Mason-Pawelek scheme of tyrosine melanin synthesis is suggested in relation to depletion of melanin in vitiligo is discussed.

- **Corsaro C, Scalia M, Sinatra F, Sichel G.**
Circannual rhythm of the melanin content in frog liver (Rana esculenta L.). **Pigment Cell Res 3:120-122, 1990.**
Abstract : The melanin content of Rana esculenta L. liver varies according to a circannual statistically significant rhythm, as shown by variance and single cosinor analysis. The maximum is found in autumn-winter, the minimum in spring-summer. The linear regression analysis shown a negative correlation between the amount of melanin and the environmental temperature.

- **Epperlein HH, Lofberg J.**
The development of the larval pigment patterns in Triturus alpestris and Ambystoma mexicanum. **Adv Anat Embryol Cell Biol 118:1-99, 1990.**
Abstract : 1. Melanophores and xanthophores are pigment cell derivatives of the NC. In amphibian embryos they migrate from their original position on the neural tube dorsally (into the dorsal fin) as well as laterally (between somites and epidermis) and arrange themselves into typical pigment patterns of the skin. We investigated pigment pattern formation in two species of tailed amphibians, Triturus alpestris (alpine newt) and Ambystoma mexicanum (Mexican axolotl). In larvae of T. alpestris alternating longitudinal stripes or bands of melanophores and xanthophores develop, whereas in larvae of A. mexicanum a barred pattern with alternating transverse bands of melanophores and xanthophores is formed. Iridophores, a third type of pigment cell, are present later in both species and therefore play no role during early larval pigment pattern development. Visibly differentiated melanophores and xanthophores can be distinguished from each other under the light microscope by their contents of black melanins and yellow pterins respectively. With the dopa reaction (indicates tyrosinase in melanophores), and ammonia treatment (stimulates pterin fluorescence in xanthophores), the pigment cell phenotypes can be visualized even before their normal visible differentiation. In the TEM, melanophores and xanthophores can be distinguished from each other by their morphologically distinct pigment organelles and in the SEM by their different surface structure. 2. Because of the NC origin of melanophores and xanthophores and the ease with which these cells can be demonstrated even before they are visible from outside, their different arrangements in Triturus and axolotl embryos offer suitable model systems for studying the migration, interaction and localization of NC derivatives in relation to specific environmental influences. The environment of NC cells are the neural tube, epidermis, somites and lateral plate mesoderm, and the subepidermal ECM, a network of collagen fibrils associated with glycosaminoglycans, proteoglycans and glycoproteins. 3. Development of the pigment pattern in T. alpestris: Melanophores and xanthophores start to leave the NC at stage 28, melanophores slightly earlier than xanthophores. Both cell types become scattered in the dorsolateral trunk. In contrast to melanophores in the axolotl, melanophores in T. alpestris cannot be demonstrated with the dopa reaction before they become visibly black. From stage 29+ onwards, melanophores start to accumulate in zones alongside the dorsal and lateral somite edges, where they form compact stripes later. Xanthophores can be demonstrated from stage 28+

onwards only with the SEM (by means of their specific surface structures) or with the fluorescence microscope (by means of their fluorescing pterins). At state 34, xanthophores become visible externally as yellow cells.

- **Grill LK, Garger SJ Jr, Sverlow GD, Erwin RL.**
Melanin production. **Eur Pat Appl, 16 pp., 1990.**
Abstract : Melanin prodn. by microorganisms in excess of 0.2 g dry wt./L of growth medium is achieved by manipulating growth medium constituents, attenuating fermn. conditions, and/or by use of genetically engineered microorganisms. Thus, Streptomyces lividans TK64 (pIJ702) was cultured on a previously described medium modified to contain K2HPO4, 0.5, MgSO4.H2O 0.2, FeSO4 0.01, casein hydrolyzate 8, tyrosine 0.3, and methionine 0.1 g/L. The yield of melanin averaged 100-300 mg/L dry wt.

- **Hirano S.**
Observations on pigment granules in the bones of silky fowls. **Arch Histol Cytol 53:89-93, 1990.**
Abstract : The distribution of melanin pigment-containing cells in the bones of both young and adult silky fowls was observed. Melanin pigment was detected not only in melanocytes which were mainly distributed in the periosteum, but also in all the other types of cells in the periosteum and bone. The continuity of the number of pigment granules in melanocytes and that in the other pigment-containing cells could not be recognized because the granules in the latter cells were much fewer than those in the former. In young fowls, the pigment-containing cells were distributed in all layers of the periosteum and bone, but their number was low. On the other hand, in aged fowls, most of the cells in the periosteum had pigment granules. In the bone, however, pigment granules were observed only in osteocyte situated near the surface. These findings suggest that the pigment granules which are observed in osteocytes have been transferred from melanocytes to osteogenic cells or osteoblasts before they differentiate to osteocytes, where they are presumed to be digested.

- **Ho TC, St J.**
Pigmented Paget's disease of the male breast. **J Am Acad Dermatol 23:338-341, 1990.**
Abstract : Paget's disease of the breast presents as an eczematous lesion of the nipple. Histologically, it is characterized by malignant intraepidermal cells associated with an underlying ductal carcinoma. Paget's disease of the male breast is rare. Although melanin has been found within the malignant cells of Paget's disease, no clinically pigmented lesion has yet been reported. We present a case of pigmented Paget's disease of the male breast and review the pathogenesis of this disease.

- **Howard RJ, Ferrari MA.**
Role of melanin in appressorium function [Erratum to document cited in CA112(9):73492c]. **Exp Mycol 14:195, 1990.**
Abstract : Cor. Figures 9-11 have been provided. The error was not reflected in the abstr. or the index entries.

- **Katsumata M, Matsunaga T, Maruyama R, Ezoe K.**
Lymphatic invasion of nevus cells observed in intradermal nevus. **J Dermatol 17:264-265, 1990.**
Abstract : A pigmented nevus was observed on the medial aspect of the left scapula of a 30-year-old woman. Histologically, the lesion showed the pattern of a typical intradermal nevus, consisting of type A, B and C cells. However, the most characteristic feature of this intradermal nevus was the projection of polypoid masses of nevus cells containing melanin into the lumen of a lymphatic vessel in the upper dermis. To our knowledge, lymphatic invasion in pigmented nevi is rare; this finding is interesting, if we consider the relationship between nevus cells and lymph nodes.

- **Kono T, Furukawa M, Tanii T, Mizuno N, Taniguchi S, Ishii M, Hamada T.**
Enhanced melanogenesis of murine melanoma cells cultured on or in collagen gel. **Arch Dermatol Res 282:263-266, 1990.**
Abstract : To elucidate the interaction between melanoma and its matrix, we cultured B16 murine melanoma cells on and in type I collagen gel and evaluated specified functions of melanoma cells; tyrosinase activity and melanin-synthesizing capacity. Proliferation of cells cultured in these environments was markedly suppressed compared with that of cells cultured conventionally on plastic. On the other hand, the tyrosinase activity of cells cultured in or on collagen gel was two to three times higher than that of cells cultured on

the plastics, while their melanin production was approximately double that achieved during conventional culture of cells. In conclusion, collagen gel influenced the growth and cell-specific functions of the melanoma cell. The culture system using collagen gel as substrate may be useful for the investigation of the interaction between melanoma and its matrix.

- **Kurbanov K.**
Tyrosine and methionine metabolism in various states ofmelaninogenesis. **Biokhimiia 55:165-172, 1990.**
Abstract : Excretion with urine of tyrosine and methionine metabolites as well as the activities of enzymes involved in their metabolism are correlated with the state and type of melanin synthesized in the skin. The response of tyrosine aminotransferase to melaninogenesis induction was more pronounced in animals with predominant pheomelaninogenesis, especially after tyrosine load, while that to dopachrome oxidoreductase-in animals with predominant eumelaninogenesis and after methionine load. Glutathione reductase and cystathionine-beta-synthase responded more vigorously to methionine injections, which was especially well pronounced in animals with prominent pheomelaninogenesis and in albino animals. The metabolic "block" in melanine synthesis in albino animals seems to be observed after the 5-S-cysteinyl-DOPA synthesis, whereas the initial steps of melaninogenesis in these animals are identical to pheomelanine synthesis reactions.

- **Lavrent'eva GI, Ovsianko AV.**
The use of the protoplast method for the selection of oleandomycin producers. **Antibiot Khimioter 35:17-20, 1990.**
Abstract : The use of protoplasting with subsequent reversion to the cellular form in improvement of the oleandomycin-producing organism provided a 110% increase in the range of culture variation with respect to the antibiotic production property. A regenerant with a potency of 12 to 20% higher than that of the initial strain which produced 30% lower amounts of dark pigments of melanin nature was isolated. Repeated protoplasting and regeneration of the regenerant provided a very low regeneration frequency i.e. 0.0002%. The potency of all the secondary regenerants was low.

- **Maeda K, Tomita Y, Tagami H.**
Melanocyte-stimulating properties of proinflammatory chemical mediators. **Ensho 10:189-194, 1990.**
Abstract : Skin darkening after cutaneous inflammation is a well known phenomenon but its mechanisms for this hyperpigmentation have not been clarified yet. Because in inflamed skin various mediators such as arachidonic acid metabolites and histamine are found in increased amts., this study investigated their effects on cultured normal human melanocytes. As reported previously the authors found that prostaglandin E2 stimulated normal human melanocytes. In addn. histamine, platelet activating factor, bradykinin and arachidonic acid metabolites such as leukotriene (LT) C4 and LTD4 also stimulated melanocytes. They were found to increase the total amts. of immunoreactive tyrosinase and tyrosinase related protein, the no. of dendrites and the size of melanocytes. Of these proinflammatory mediators, LTC4 and histamine showed the strongest stimulatory effects. On the other hand, serotonin, heparin and other arachidonic acid metabolites such as PGE1, PGFs, and 12-hydroxy eicosatetraenoic acid (12-HETE) did not show any stimulatory effect. The results suggest that various proinflammatory chem. mediators, esp. LTC4 and histamine are involved in the stimulation of melanocytes to accelerate the prodn. of melanin and its active transfer to neighboring keratinocytes, resulting in the formation of hyperpigmentation after skin inflammation.

- **Nikolaev ON, Aver'yanov AA.**
Effect of the fungicide tricyclazole on pigmentation of the fungus Piricularia oryzae Cav. during early stages of development. **Agrokhimiya 110-115, 1990.**
Abstract : Growing P. oryzae spores in media contg. 0.5 .mu.g tricyclazole/mL, followed by rinsing from tricyclazole, prevented development of compatible blast spots on rice leaves inoculated with (0.6-2.5) .times. 103 spores/mL. At (10-75) .times. 103 spores/mL the effectiveness of tricyclazole gradually decreased. Growing spores with tricyclazole partly suppressed repigmentation of spores whose pigment normally decreases within the 1st 24 h of germination and then is partly restored. The transient pigmentation decrease during spore germination was caused by loss of melanin which was reconstituted in appressoria. Thus, the action of melanin-inhibiting fungicides may be assocd. with impairment of repigmentation during spore germination.

- **Obika M, Meyer-Rochow VB.**
Dermal and epidermal chromatophores of the Antarctic teleost Trematomus bernacchii. **Pigment Cell Res 3:33-37, 1990.**
Abstract : The physiological response and ultrastructure of the pigment cells of Trematomus bernacchii, an Antarctic teleost that lives under the sea ice north of the Ross Ice Shelf, were studied. In the integument, two types of epidermal chromatophores, melanophores and xanthophores, were found; in the dermis, typically three types of chromatophores --melanophores, xanthophores, and iridophores-- were observed. The occurrence of epidermal xanthophore is reported for the first time in fish. Dermal melanophores and xanthophores have well-developed arrays of cytoplasmic microtubules. They responded rapidly to epinephrine and teleost melanin-concentrating hormone (MCH) with pigment aggregation and to theophylline with pigment dispersion. Total darkness elicited pigment aggregation in the majority of dermal xanthophores of isolated scales, whereas melanophores remained dispersed under both light and dark conditions. Pigment organelles of epidermal and dermal xanthophores that translocate during the pigmentary responses are carotenoid droplets of relatively large size. Dermal iridophores containing large reflecting platelets appeared to be immobile.

- **Papp P.**
Changing reddish brown color to bluish gray in fine furs. **Bor- Cipotech, 40(1):14-16, 1990.**
Abstract : The reddish brown color of fine furs (e.g., muskrat) is changed to a more desirable bluish grey by having the melanin pigment of the hair bind divalent Fe from Fe salt solns. The bound divalent Fe cannot be removed and the resistance to fading is also increased for the furs.

- **Park C, Cho NH, Jeong HJ.**
Melanosis coli--histochemical and immunohistochemical comparison of the pigments of melanosis coli and Dubin-Johnson syndrome. **Yonsei Med J 31:27-32, 1990.**
Abstract : We compared the pigment of melanosis coli with the pigment of Dubin-Johnson syndrome, melanin, and lipofuscin. The pigment of melanosis coli appeared similar to lipofuscin in that it stained positively with periodic acid-Schiff, oil red-O and Victoria blue stains and revealed negative reactions to the immunohistochemical stains for S-100 protein and neuron specific enolase, but had similarity to melanin as shown by the positive reaction to Fontana-Masson stain and negative autofluorescence. The pigment of Dubin-Johnson syndrome showed the same histochemical and immunohistochemical characteristics as that of melanosis coli. The results indicate that the pigments of melanosis coli and Dubin-Johnson syndrome are identical and are variants of lipofuscin.

- **Pintucci G, Manzionna MM, Maida I, Boffi M, Boffoli D, Gallone A, Cicero R.**
Morpho-functional characterization of cultured pigment cells from Rana esculenta L. liver. **In Vitro Cell Dev Biol 26:659-664, 1990.**
Abstract : A simple method to isolate and culture liver pigment cells from Rana esculenta L. is described which utilizes a pronase digestion of perfused liver, followed by sedimentation on a Ficoll gradient. A first characterization of isolated and cultured cells is also reported. They show both positivity for nonspecific esterases, and phagocytosis ability, like the cells of phagocytic lineage. Furthermore, after stimulation with a phorbol ester, these cells generate superoxide anions. At phase contrast microscope, liver pigment cells present variability in size, morphology, and in their content of dark-brown granules. In as much as a cell extract obtained from cultured cells exhibits a specific protein band with dopa-oxidase activity, when run on nondenaturing polyacrylamide gel electrophoresis, liver pigment cells from Rana esculenta L. should not be considered as melanophages, but as cells that can actively synthesize melanin. The method presented here seems to be useful to more directly investigate this extra-cutaneous melanin-containing cell system and to clarify its physiologic relevance.

- **Scalia M, Geremia E, Corsaro C, Santoro C, Baratta D, Sichel G.**
Lipid peroxidation in pigmented and unpigmented liver tissues: protective role of melanin. **Pigment Cell Res 3:115-119, 1990.**
Abstract : The protective role of melanin as an antioxidant biopolymer against lipid peroxidation was investigated. In pigmented frog liver and in albino rat liver the following were tested: thiobarbituric acid (TBA) reactive material (to show the induced lipoperoxidation in vitro), fatty acids, and reduced glutathione

content. Our results show that susceptibility to the in vitro lipoperoxidation induced by ferrous ions is lower in the tissue containing melanin, though the content of the polyunsaturated fatty acids is higher in pigmented than in unpigmented tissues and reduced glutathione levels are lower in pigmented tissue. Our data support the hypothesis that melanin could reduce lipoperoxidation in pigmented tissue.

- **Schulten EA, Jovanovic A, van der Waal I.**
Prevalence study of oral mucosal lesions in 300 patients. **Ned Tijdschr Tandheelkd 96:538-539, 1989.**
Abstract : Intraoral examination was performed in 300 consecutive patients, who attended the Department of Oral and Maxillofacial Surgery of the Free University Hospital, Amsterdam. In 89% of the patients one or more oral mucosal lesions were observed. Fordyce's spots, coated tongue, leukoedema, melanin pigmentation, frictional keratosis, and morsicatio buccarum were the most frequent occurring lesions.

- **Slominski A, Paus R.**
Are L-tyrosine and L-dopa hormone-like bioregulators? **J Theor Biol 143:123-138, 1990.**
Abstract : Some amino acids have bioregulatory functions, which far exceed those of precursors for proteins or of substrates for specific enzymes. Two of these amino acids, L-tyrosine and L-dopa, are precursors to melanin and catecholamines. In vertebrates, they can act as inducers and regulators of the melanogenic apparatus and of MSH receptors--two quite complex functions that could hardly be performed by mere substrates. Focussing on the pigmentary system as a study model, we therefore explore the hypothesis that L-tyrosine and L-dopa act as hormone-like bioregulators in mammals, with melanocytes regulating tyrosine and dopa activity via their metabolic consumption.

- **Suzuki H, Okazawa T, Kere N, Kawada H.**
Field evaluation of a new insect growth regulator, pyriproxyfen, against Anopheles farauti, the main vector of malaria in the Solomon Islands. **Eisei Dobutsu 40:253-257, 1989.**
Abstract : A field study to control the malaria vector, A. farauti, with an insect growth regulator, pyriproxyfen (S-31183; I), was carried out in northern Guadalcanal in the Solomon Islands. Am emulsifiable conc. of 1% pyriproxyfen was applied to two stagnant and flowing breeding sites: one with fresh water and another with brackish water. Pyriproxyfen at 0.1 inhibited emergence of A. farauti completely, at both test sites, for at least 5 wk after treatment and the efficacy (more than 70% inhibition) lasted for ca. 2 mo. The body color of the larvae and pupae in the test sites whitened noticeably after the application of the compd. This is probably the 2st report on inhibition of melanization in mosquito larvae.

- **Szekeres E, Korom I, Zombai E.**
Hyperpigmentation of the face. **Hautarzt 41:164-167, 1990.**
Abstract : A 49-year-old male patient is presented, who developed hyperpigmented macules on the face. An exact classification of the disorder was not possible on the basis of anamnestic data, histology and electron microscopy. An attempt was made to differentiate it from other known dyschromias of the face.

- **Tobin DJ, Fenton DA, Kendall MD.**
Ultrastructural observations on the hair bulb melanocytes and melanosomes in acute alopecia areata. **J Invest Dermatol 94:803-807, 1990.**
Abstract : It is well recognized that alopecia areata (Aa) may preferentially affect pigmented hair and may spare white hair, and that regrowing hair in the disease is often initially white. In addition, there is an association with vitiligo and ocular depigmentation. To date, the pathomechanisms of the melanocyte effects are unclear. We have studied 10 patients with untreated acute alopecia areata, and three normal patients without hair loss. Morphologic changes, studied by conventional light and electron microscopy, in the cytoplasm of affected melanocytes often predated nuclear hyperchromatism. Increased numbers of bizarre melanosomes were found in affected melanocytes compared with normal ones; such melanosomes had incomplete or "aborted" melanization, resulting in poor pigment deposition, and were disrupted, enlarged and rounded, with loss of normal ellipsoidal shape. An unusual outer root sheath (ORS) distribution of hair bulb melanocytes was seen. Other atypical melanosome effects included marked pigment displacement into peribulbar and DP melanophages. In the DP clumped melanin granules formed giant spherical complexes without discernible limiting membranes, which were sometimes associated with lymphocytes. These morphologic changes indicate an active involvement of hair bulb melanocytes in alopecia areata.

- **Wright AL, Bleehen SS, Champion AE.**
Reticulate pigmentation due to bleomycin: light- and electron-microscopic studies. **Dermatologica 180:255-257, 1990.**
Abstract : An unusual case of extensive reticulate pigmentation due to bleomycin is reported. Light- and electron-microscopic studies showed a marked increase of melanin pigment in the basal keratinocytes and a number of melanophages in the upper dermis. The number of melanocytes appeared to be normal. Electron microscopy also revealed damage to subcellular organelles. The increase in pigmentation is thought to be a consequence of this focal damage.

3. MSH, MCH, other hormones, differentiation

- **al-Obeidi F, Hruby VJ, Hadley ME, Sawyer TK, Castrucci AM.**
Design, synthesis, and biological activities of a potent and selective alpha-melanotropin antagonist. **Int J Pept Protein Res 35:228-234, 1990.**
Abstract : Based on structure-activity relationships of the potent alpha-MSH agonist, Ac-Nle4-Asp5-His6-D-Phe7-Arg8-Trp9-Lys10-NH2, several analogs of the general formula Ac-Nle4-Asp5-Waa6-Xaa7-Yaa8-Zaa9-Lys10+ ++-NH2 were synthesized and tested on frog and lizard skin bioassays for their possible inhibitory actions against alpha-MSH on melanocyte stimulation. When Waa6 = Trp, Xaa7 = D-Phe, Yaa8 = Nle and Zaa = Trp, a highly potent alpha-MSH antagonist, Ac-Nle-Asp-Trp-D-Phe-Nle-Trp-Lys-NH2, with selectivity on the frog skin alpha-MSH receptor system (pA2 = 8.4) was obtained. However, several modifications in the amino acid sequence of the peptide resulted in a complete loss of antagonistic activity and a recovery of very weak agonistic action. The following changes in the amino acid sequence of the peptide were examined; His or D-Trp for Waa, L-Phe for Xaa, Arg, Ala or Pro for Yaa, and D-Trp for Zaa. All resulted in full agonists with no antagonistic activity. In addition, lactam cyclization between the Asp5 and Lys10 side chains in the antagonist gave a full agonist and a complete loss of antagonistic activity. Efforts to develop a rational approach for the design of selective alpha-MSH antagonists for the frog skin alpha-MSH receptor will be discussed.

- **Bird DJ, Baker BI, Eberle A, Swann RW.**
The biosynthesis of melanin-concentrating hormone in a fish. **J Neuroendocrinol 2:309-315, 1990.**
Abstract : The biosynthesis of melanin-concg. hormone (MCH) was investigated in the trout (Salmo gairdneri). Sephadex G-75 chromatog. showed the presence of several large MCH-immunoreactive mols. in hypothalamic and pituitary gland exts., with retention times on HPLC different from the mature MCH1-17. About 10% of the total MCH immunoreactivity in the hypothalamus was attributable to large-mol.-wt. forms, but these contributed <1% to the immunoreactivity in the neurointermediate lobe. Both [35S]methionine and [3H]leucine were injected into the hypothalamus near the MCH perikarya (nucleus lateralis tuberis region) of anesthetized fish, after which the fish were killed at intervals of .ltoreq.8 h postinjection, and the basal hypothalami, pituitary pars distales, and neurointermediate lobes were extd. in acid. MCH-related immunoppts. from these exts. were fractionated by SDS-PAGE or by Sephadex G-50 chromatog. Radiolabel was incorporated into 15.3- and 11.3-kilodalton precursors within 0.75 h, which were converted, via several smaller intermediates, to a mol. resembling MCH1-17. The results are discussed in relation to the known cDNA sequence of salmon MCH. Labeled MCH first appeared in the neurointermediate lobe 4 h after injection, giving an estd. transit rate of 0.4 mm/h.

- **Friedmann PS, Wren F, Buffey J, Macneil S.**
Alpha-MSH causes a small rise in cAMP but has no effect on basal or ultraviolet-stimulated melanogenesis in human melanocytes. **Br J Dermatol 123:145-151, 1990.**
Abstract : The effects of alpha-melanocyte stimulating hormone (alpha-MSH) were studied on levels of cyclic adenosine 3',5'-monophosphate (cAMP), melanin content and response to ultraviolet radiation (UVR) in cultured human melanocytes (HuMC). Foreskin HuMC were cultured in a hormone-supplemented system not dependent on the presence of phorbol esters. Following addition of alpha-MSH (10(-6) M) there was a rise in cAMP levels maximal between 5 and 15 min to 9.4 +/- 3.2 pM/10(5) cells, while control levels were 3.6 +/- 0.7 pM/10(5) cells. After 7 days' culture in the presence of alpha-MSH (10(-8) -10(-6) M) the melanin content increased by only 35%, whereas Forskolin (10(-5) M) induced a 9.5-fold rise in cAMP after 5 min

and a 10.9-fold rise in melanin content after 7 days. When HuMC were irradiated daily for 6 days with UVR (Helarium fluorescent lamps emitting 20% UVB, 80% UVA) melanin content rose 2.7-fold (SE 0.3). This was unchanged or slightly reduced in the presence of alpha-MSH (10(-8)-10(-6) M). Parallel observations on Cloudman S91 melanoma cells showed that alpha-MSH caused only an 80% increase in melanin content after 4 days. The rise in melanin content induced by three daily UV-irradiations (2.4-fold, SE 0.5) was unchanged by alpha-MSH (10(-8)-10(-6) M). Although alpha-MSH induces a small rise in cAMP in HuMC this does not result in melanogenesis, and the response to UVR is not affected by alpha-MSH in either HuMC or S91 cells.

- **Kameyama K, Vieira WD, Tsukamoto K, Law LW, Hearing VJ.**
Differentiation and the tumorigenic and metastatic phenotype of murine melanoma cells. **Int J Cancer 45:1151-1158, 1990.**
Abstract : Using B16 F10 murine melanoma cells and sublines generated from the JB/MS melanoma which exhibit various degrees of melanogenesis, the relationships among differentiation, tumorigenicity, and metastatic potential were examined. The effect of melanocyte-stimulating hormone (MSH), which specifically stimulates differentiation of melanocytes, was also studied. All melanoma lines tested were capable of growing as experimental pulmonary metastases but, surprisingly, the undifferentiated and amelanotic JB/MS-w cells failed to grow as primary subcutaneous tumors. JB/MS-w cells, which had few surface MSH receptors, did not respond to MSH with an increase in melanin production, unlike the other cell lines. Although in vitro treatment with MSH did not change the rates of growth of primary tumors by these cell lines, such treatment decreased the number of pulmonary metastases from B16 F10, JB/MS cells, JB/MS-b1 cells and JB/MS-w cells. Conversely, MSH treatment significantly increased the rates of pulmonary metastases from JB/MS-p cells. The expression of surface melanoma antigens, urokinase-type plasminogen activity and susceptibility to natural killer cells were examined. MSH did not significantly alter surface melanoma antigen expression, but increased the natural killer cell susceptibility of B16 F10, JB/MS and JB/MS-b1 cells, cells which possess abundant surface MSH receptors. There was an inverse correlation between differentiation (pigmentation) and proliferation in vitro, and the more pigmented melanoma cells (B16 F10, JB/MS and JB/MS-b1) expressed relatively lower levels of class-I MHC, relatively higher levels of class-II MHC and the highest metastatic capacity. These results demonstrate that MSH possesses the capacity to regulate not only melanogenesis, but also other factors critical to the metastatic growth of the cells.

- **Raina AK, Jaffe H, Kemp TG.**
Peptides stimulating sex pheromone production and melanization in moths. **US Pat Appl, 19 pp., 1989.**
Abstract : A pheromone biosynthesis activating neuropeptide (Hez-PBAN) hormone, controlling sex pheromone prodn. in moths and controlling melanizing in larvae, was isolated from the brain-subesophageal ganglion complexes of adult corn earworm (Heliothis zea). Hez-PBAN has 33 amino acid residues and a mol. wt. of 3900; its amino acid sequence is unique among the fully characterized peptide hormones. Synthetic PBAN and related structures induced prodn. of sex pheromone in ligated H. zea females and other moth species and melanization in larvae, that resulted in morphol. changes or death.

4. Photobiology and photochemistry

- **Andersen PH, Bjerring P.**
Spectral reflectance of human skin in vivo. **Photodermatol 7:5-12, 1990.**
Abstract : A newly developed skin reflectance spectrophotometer was evaluated for measurements of both melanin pigmentation and erythema. Physiological changes in blood flow and blood content in normal humans were induced by compression with an arm cuff during recording of skin reflectance spectra. Reflectance spectra of UV-induced erythema were also recorded and compared with laser-Doppler flow measurements. Spectral reflectance measurements were found to be highly sensitive in determining minimal erythema, which was not clinically detectable. The measurements of erythema using reflectance spectroscopy and UV irradiation were very highly correlated ($r = 0.996$). It was possible to calculate the in vivo absorbance of oxygenized haemoglobin. The melanin pigmentation following UV irradiation was quantified by reflectance spectroscopy and correlates highly with the dose of UV irradiation ($r = 0.995$). Furthermore, regional variations in skin melanin and haemoglobin were analysed for fair Caucasian skin.

- **Gallas JM.**
Medium incorporating melanin as an absorbing pigment for protection against electromagnetic radiation. **PCT Int Appl, 61 pp., 1990.**
Abstract : Electromagnetic (e.g., UV, visible, and near IR) radiation-absorbing app. comprises a solid material assocd. with melanin. The app. may comprise ophthalmic devices, sunglasses, contact lenses, intraocular devices, packaging, a plastic film, windows, umbrellas, or canopies. The melanin may be present in a coating or it may be incorporated within the solid material. Articles with assocd. melanin and methods of forming them are described.

- **Harber LC, DeLeo VA, Prystowsky JH.**
Intrinsic and extrinsic photoprotection against UVB and UVA radiation. **Cosmet Sci Technol Ser 10:359-378, 1990.**
Abstract : A review with 48 refs. on endogenous skin components (keratin, melanin, pigments), exogenous phys. barriers (ozone, pollutants, clothing, water), and sunscreens for resin photoprotection against UVA and UVB radiation.

- **Huselton CA, Hill HZ.**
Melanin photosensitizes ultraviolet light (UVC) DNA damage in pigmented cells. **Environ Mol Mutagen 16:37-43, 1990.**
Abstract : Melanins, pigments of photoprotection and camouflage, are very photoreactive and can both absorb and emit active oxygen species. Nevertheless, black skinned individuals rarely develop skin cancer and melanin is assumed to act as a solar screen. Since DNA is the target for solar carcinogenesis, the effect of melanin on Ultraviolet (UV)-induced thymine lesions was examined in mouse melanoma and carcinoma cells that varied in melanin content. Cells prelabeled with 14C-dThd were irradiated with UVC; DNA was isolated, purified, degraded to bases by acid hydrolysis and analyzed by HPLC. Thymine dimers were detected in all of the extracts of irradiated cells. Melanotic and hypomelanotic but not mammary carcinoma cell DNA from irradiated cells contained hydrophilic thymine derivatives. The quantity of these damaged bases was a function of both the UVC dose and the cellular melanin content. One such derivative was identified by gas chromatography-mass spectroscopy as thymine glycol. The other appears to be derived from thymine glycol by further oxidation during acid hydrolysis of the DNA. The finding of oxidative DNA damage in melanin-containing cells suggests that melanin may be implicated in the etiology of caucasian skin cancer, particularly melanoma. Furthermore, the projected decrease in stratospheric ozone could impact in an unanticipated deleterious manner on dark-skinned individuals.

- **Jeffery WR.**
Ultraviolet irradiation during ooplasmic segregation prevents gastrulation, sensory cell induction, and axis formation in the ascidian embryo. **Dev Biol 140:388-400, 1990.**
Abstract : The effect of ultraviolet (uv) light on embryonic development was examined in the ascidian Styela clava. uv irradiation (3.0 x 10(-3) J mm-2) of the entire surface of fertilized eggs during ooplasmic segregation prevented gastrulation, sensory cell induction, and embryonic axis formation. The uv-irradiated embryos completed ooplasmic segregation and cleaved normally, but vegetal blastomeres did not invaginate at the beginning of gastrulation, sensory cells in the larval brain did not develop tyrosinase or melanin pigment, and the larval tail did not develop. Endoderm, epidermis, and muscle cells differentiated in the uv-irradiated embryos, however, as evidenced by expression of endodermal alkaline phosphatase (AP), an epidermal-specific antigen, and alpha-actin, myosin heavy chain, and acetylcholinesterase (AChE) in muscle cells. Higher doses of uv light (6.0-9.0 x 10(-3) J mm-2) suppressed expression of the epidermal antigen and muscle cell markers, whereas the development of endodermal AP was insensitive. Irradiation at various times between fertilization and the 16-cell stage revealed that gastrulation, sensory cell differentiation, and axis formation are sensitive to uv light only during ooplasmic segregation. Irradiation of restricted regions of the zygote during ooplasmic segregation showed that the uv-sensitive components are localized in the vegetal hemisphere. The absorption characteristics of the uv-sensitive components suggest that they are nucleic acids. The results show that uv-sensitive components that specify gastrulation, sensory cell induction, and embryonic axis formation are localized in the vegetal hemisphere of Styela eggs.

- **Jori G, Spikes JD.**
Photothermal sensitizers: possible use in tumor therapy. **J Photochem Photobiol B, 6(1-2):93-101, 1990.**
Abstract : A review with 27 refs. Photothermal damage of tissues or endotissular compartments may be induced by pulsed irradn. of either endogenous chromophores (e.g., Hb, melanin) or externally added dyes; the latter should have short triplet lifetimes and mainly decay from electronically excited states by nonradiative pathways. Potential photothermal sensitizers are some metallo derivs. of porphyrins and porphyrinoid compds., azo dyes and triphenylmethane derivs. These dyes have the addnl. property of significant absorbance at wavelengths >600 nm, which can penetrate deep into biol. tissues. Spatial confinement of the photothermal process depends on the absorption coeff. of the photoexcited chromophore and its thermal reaction time. Present evidence indicates that the selective photothermal damage of macromols. or subcellular organelles requires pulsed excitation at picosecond or nanosecond regimes, whereas microsecond or millisecond domains are effective in the case of cells or similar structures. The possible use of photothermal sensitization in the treatment of tumors is briefly discussed.

- **Matsuoka LY, Wortsman J, Hollis BW.**
Suntanning and cutaneous synthesis of vitamin D3. **J Lab Clin Med 116:87-90, 1990.**
Abstract : Skin tanning is the melanization of the epidermis induced by excessive sunlight exposure. Since melanin absorbs preferentially the wavelengths around 300 nm and the cutaneous synthesis of vitamin D3 is stimulated by the same wavelengths (290 to 320 nm, ultraviolet light B [UVB]), we investigated the effect of tanning on vitamin D3 formation. Vitamin D3 and 25 hydroxyvitamin D (25-OH-D) serum levels were measured during midwinter (untanned state) in seven healthy subjects. Blood was obtained immediately before whole body exposure to UVB in a phototherapy unit, and again 24 hours later. The study was repeated in the same subjects during midsummer (tanned state) using the same UVB dose. Serum vitamin D3 increased in the untanned state from 1.7 +/- 0.4 ng/ml (mean +/- SE) to 11 +/- 1.5 ng/ml following UVB ($p < 0.001$). In the tanned state, basal serum vitamin D3 was significantly higher: 9.6 +/- 2.8 ng/ml ($p < 0.04$ basal untanned versus basal tanned), and exhibited minimal rise after UVB to 14.3 +/- 4.1 ng/ml ($p > 0.1$ for tanned basal versus post UVB tanned). Tanning was also associated with significantly higher serum 25-OH-D levels: 22.5 +/- 2.9 ng/ml (untanned) versus 36.9 +/- 4.7 ng/ml (tanned) ($p < 0.02$). Thus excessive solar exposure produces, besides erythema and tanning, the resetting of the vitamin D3 synthetic mechanism with blunting of the response to UVB.

- **Niggli HJ.**
Comparative studies on the correlation between pyrimidine dimer formation and tyrosinase activity in Cloudman S91 melanoma cells after ultraviolet-irradiation. **Photochem Photobiol 52:519-524, 1990.**
Abstract : The induction of pyrimidine dimer d. was studied after UV-irradn. in mouse melanoma cells before and after treatment with cholera toxin. Treatment with cholera toxin stimulated tyrosinase activity up to 50-fold, leading to a marked, visually apparent increase in cellular melanin concns. Irradn. of treated and untreated cells was therefore designed to establish whether intracellular melanin protected cells from UV-induced DNA damage. In expts. described here, cytosine-thymine (C-T) as well as thymine dimer levels (T-T) were detd. by HPLC in cholera toxin-treated and untreated Cloudman S91 mouse melanoma cells after irradn. with UVC (<290 nm) and UVB light (290-320 nm). Surprisingly, induction of melanization had no effect on the formation of pyrimidine dimers by UVC or UVB irradn. These results indicate than de novo melanin pigmentation induced via the cAMP pathway is not involved in protection against UV-induced thymine-contg. pyrimidine dimers. In sep. expts., irradn. of toxin-treated and untreated mouse melanoma cells with UVC or UVB light produced a 20-30% lower dimer d. compared to irradiated human skin fibroblasts. This finding suggests that melanin has some protection properties against UV-induced pyrimidine dimers, although the exact defense mechanism seems highly complex.

- **Pierce-Liebisch JA.**
Diffuse reflectance infrared spectrometry of base-solubilized fungal melanin [Erratum to document cited in CA111(25):227523g]. **Physiol Chem Phys Med NMR 21:243, 1989.**
Abstract : Table I, omitted from the original article, has been provided. The error was not reflected in the abstr. or the index entries.

- **Rosen CF, Jacques SL, Stuart ME, Gange RW.**
Immediate pigment darkening: visual and reflectance spectrophotometric analysis of action spectrum. **Photochem Photobiol 51:583-588, 1990.**
Abstract : Immediate pigment darkening (IPD) occurs in human skin upon exposure to ultraviolet-A and visible radiation. The spectral changes that occur during IPD were measured with a rapid scanning reflectance spectrophotometer (RS) which employs optical fiber bundles for delivery and detection of light between 400 and 750 nm. The radiation dose dependence and wavelength dependence (334-549 nm irradiation) of IPD were studied by both the classical visual grading method and by spectrophotometric scoring using the RS system. The spectral changes that occur at long wavelengths with IPD mimic the natural absorption spectrum of melanin. Therefore, the IPD was scored in terms of the apparent change in melanin optical density, using the method Kollias and Baqer [Photochem. Photobiol. 43:49-54 (1986)], based on reflectance in the 620-720 nm range. The nonlinearity of the visual grading method is demonstrated. The degree of IPD is first-order with respect to delivered dose and saturates after high doses. The maximum amount of IPD attained at saturation is greater for shorter wavelengths. Extrapolation of the reflectance data suggests the longest wavelength capable of eliciting IPD is about 470 nm.

5. Neuromelanins

- **Arima K, Akashi T.**
Involvement of the locus coeruleus in Pick's disease with or without Pick body formation. **Acta Neuropathol (Berl) 79:629-633, 1990.**
Abstract : Brains affected by the fronto-temporal type of Pick's disease were classified into two subgroups according to whether Pick bodies (PBs) were detectable in cerebral cortex (PB-positive group, six cases) or not (PB-negative group, eight cases), and examined neuropathologically. Controls included seven patients with non-degenerative diseases. The neuronal population in the locus coeruleus (LC) was estimated quantitatively in preparations from the middle part of the LC. The data were analyzed statistically by the Mann-Whitney U-test. Histological and ultrastructural studies were also carried out. The following results were obtained: (1) there were no appreciable differences between the PB-positive and PB-negative groups with regard to age at onset, age at death, duration of illness, clinical stage at death, and brain weight; (2) the mean nerve cell counts in the LC were 43.7 +/- 5.2 in the controls, 28.8 +/- 11.7 in the PB-positive group, and 42.9 +/- 7.6 in the PB-negative group. The nerve cell count in the PB-positive group was significantly lower (P less than 0.05) than those in the controls and the PB-negative group; and (3) in each of the PB-positive cases, PBs were disclosed in the LC, in medium-sized melanin-laden neurons and small neurons. PBs were globular or lobulated, and their fine structure was identical to that of typical PBs in the cerebral cortex. In conclusion, PB formation may play an important role in neuronal decrease in the LC of PB-positive cases, whereas the LC may not be affected in PB-negative cases. In this respect, Pick's disease with PB formation appears distinct from that without PB formation.

- **Barrenas ML, Lindgren F.**
The influence of inner ear melanin on susceptibility to TTS in humans. **Scand Audiol 19:97-102, 1990.**
Abstract : In order to investigate the function of the inner ear melanin, the relationship between skin pigmentation and noise-induced temporary hearing loss (TTS) was studied. Forty-four normal-hearing Caucasian subjects were divided into three groups according to their sun sensitivity. Hearing thresholds before and after exposure were ascertained with a computerized sweep frequency audiometer in the frequency range 2-8 kHz. The noise exposure consisted of a 1/3-octave band-filtered noise with a centre frequency of 2 kHz at 105 dB SPL for 10 min. The mean TTS in the frequency range 2-8 kHz showed statistically significant differences between the three groups, i.e. the most pigmented subjects developed least TTS, and the least pigmented subjects most TTS.

- **Bednar B.**
Melanotic paraneurons. **Cesk Patol 26:3-8, 1990.**
Abstract : Histogenetic scheme of three germ layers causes difficulties in explanation of some tumorous structures derived from different classes. A conception of paraneurons concedes (besides "central" neuroectodermal paraneurons) a possibility of their local origin. The idea anticipates a broader phylogenetic

ability of divergent differentiation of single extraneural cells. Paraneuronal theory used in oncology can explain local origin of some presumable combination variants of tumours, e.g. carcinoid type paraneuromas with melanin pigmentation. Substantial frequency of such tumours eliminates possibility of random coincidence and argues for ranging "peripheral melanin system" among paraneurons.

- **Ben-Shachar D, Youdim MB.**
Selectivity of melaninized nigra-striatal dopamine neurons to degeneration in Parkinson's disease may depend on iron-melanin interaction. **J Neural Transm Suppl 29:251-258, 1990.**
Abstract : The recent studies on the chemical pathology of Parkinson's disease show selective increases of iron and lipid peroxidation and decreased glutathione (GSH) oxidizing capacity in the substantia nigra (SN). These changes are indicative of oxidative stress, possibly due to the accumulation of iron in the SN. It is the melaninized dopamine neurons that are vunerable to degeneration. The investigation of the interaction of iron with dopamine melanin demonstrates the presence of two relatively high affinity binding sites for 59Fe3+ on dopamine melanin. Interaction of Fe3+ with dopamine melanin results in potentiation of lipid peroxidation of rat cerebral cortex as compared to that induced by Fe3+. Only compounds with the ability to chelate iron are able to inhibit the binding of Fe3+ to melanin and the resultant lipid peroxidation. Therapeutic use of iron chelators, with the ability of crossing the blood brain barrier, as agents for retarding the oxidative stress and Parkinson's disease is envisaged.

- **Berliner DL, Erwin RL, McGee DR.**
Prophylaxis and treatment of nervous system diseases with melanin. **PCT Int Appl, 74 pp., 1990.**
Abstract : Melanin-deficiency-related diseases of the nervous system (e.g., xeroderma pigmentosum, Parkinson's disease, senile dementia, Huntington's chlorea) are treated by the administration of melanin, its derivs., tyrosinase, tyrosinase gene, melanin-concg. hormone, or combinations thereof. Such treatment also protects against the effects of toxic substances capable of being chelated or scavenged (e.g., Al or O2-).

- **Gotz ME, Freyberger A, Riederer P.**
Oxidative stress: a role in the pathogenesis of Parkinson's disease. **J Neural Transm Suppl 29:241-249, 1990.**
Abstract : The degeneration of nigro-striatal dopaminergic neurons is considered to be a predominant pathogenetic factor of Parkinson's disease (PD). However, the etiology of this degeneration is not known. Hypotheses assume accumulation of endogenous and/or exogenous toxins as trigger of the disease. An increase in the concentration of free radicals has been suggested to be toxic to cells, especially when combined with certain metals like free iron or copper. The role of melanin in the degenerative process is not clear, but autoxidative reactions such as the oxidation of dopamine (DA) to melanin generating radicals and toxic metabolites seem to enhance the vulnerability of neurons in the substantia nigra (SN). Disappearance of melanin in the SN, increase of total iron and ferric iron, extreme decrease of glutathione (GSH) levels, reduced activity of enzymes involved in the detoxification of hydrogen peroxide, hydroxyl and superoxide radicals (peroxidases, catalase, glutathione peroxidase), an increase of monoamine oxidase B (MAO B) activity and the substantial increase of malondialdehyde, a marker of lipid peroxidation, in the SN seem to indicate a role of an oxidative stress syndrome in the SN causing or aggravating PD.

- **Rabey JM, Hefti F.**
Neuromelanin synthesis in rat and human substantia nigra. **J Neural Transm Park Dis Dement Sect 2:1-14, 1990.**
Abstract : A relation between neuromelanin synthesis and vulnerability of dopaminergic neurons is suggested by the fact that heavily pigmented cells are preferentially lost in aging and Parkinson's disease and that the dopaminergic neurotoxin MPP+ (1-methyl-4-phenyl-pyridine) binds to neuromelanin. To elucidate the mechanism of neuromelanin synthesis, we studied the formation of melanin in homogenates of human and rat substantia nigra tissue "in vitro". It was found that enzymatic processes accounted for 70% and 90% of the melanin formation in homogenates of human and rat tissue, respectively. The enzymatic synthesis was due to the activity of monoamine oxidase (MAO), since it was prevented by selective inhibitors of this enzyme. Both MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP+ inhibited melanin formation, probably due to their ability to inhibit MAO. No evidence was found for involvement of cytochrome P-450 monooxigenases, which have been postulated to exist in central catecholaminergic neurons. Proadifen reduced melanin formation, not necessarily because it is an inhibitor of P-450 monooxigenases, but rather

as it is also a potent inhibitor of MAO. Some antioxidants like ascorbic acid, but not agents destroying hydrogen peroxide, inhibited melanin formation. The findings suggest that the formation of neuromelanin in the substantia nigra involves MAO and non-enzymatic oxidative processes.

- **Sokolowski AL, Larsson BS, Lindquist NG.**
Distribution of 1-(3H)-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (3H-MPTP) in the frog: uptake in neuromelanin. **Pharmacol Toxicol 66:252-258, 1990.**
Abstract : The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes selective destruction of pigmented monoaminergic neurons of the brain, mainly in the substantia nigra. Primates and amphibians, whose nerve cells contain melanin, have shown a higher sensitivity for the toxic effects of MPTP than species which are lacking neuromelanin, e.g. rodents. In the present study the distribution after intraperitoneal injection of 3H-MPTP in frogs (Rana temporaria) was studied by whole-body autoradiography. Histochemical staining methods for melanin were used in order to identify the pigment in various tissues. Melanin-containing nerve cells were present bilaterally in the ventral motor parts of the frog brain. Melanin was also found in the meninges, around the cerebral ventricles and the aqueducts, and in the eyes, skin and liver. The results from the autoradiographic study of 3H-MPTP revealed a high accumulation and retention in all melanin-containing structures up to 15 days after administration (the longest survival time). The pigmented tissues showed the highest concentration of radioactivity in the body at all survival times. The MPTP-induced destruction of pigmented nerve cells may be related to the binding and storage of MPTP and/or its metabolites in neuromelanin, causing toxic cytoplasmic concentrations through the continuous release of substance from the melanin depot.

6. Genetics

- **Barbour WM, Elkan GH.**
Physiological characteristics and competitive ability of plasmid-cured derivatives of Rhizobium fredii USDA 206. **Arch Microbiol 154:1-4, 1990.**
Abstract : R. fredii USDA 206 carries four plasmids which total more than 1200 MDa of DNA. A series of plasmid-cured mutants of strain USDA 206 was derived and compared to det. possible functions of the plasmids, as well as the effect of the plasmids on growth and competitiveness of their host strains. No functions of plasmids pRj206a or pRj206c were found. Plasmid pRj206b had a higher copy no. in the non-mucoid (Muc-) deriv. strain 206CANS. Transfer of pRj206b conferred on two recipient strains a Muc- phenotype, indicating control of exopolysaccharide synthesis by this plasmid. The same plasmid appeared to encode repression of melanin synthesis. Strain 206CANS was shown to have a shorter generation time than USDA 206 and to out-compete USDA 206 in batch and chemostat culture. Competition for nodulation indicated little difference between USDA 206 and 206CANS, while USDA 206 appeared to be more competitive than two of the other cured derivs.

- **Beermann F, Ruppert S, Hummler E, Bosch FX, Muller G, Ruther U, Schutz G.**
Rescue of the albino phenotype by introduction of a functional tyrosinase gene into mice. **EMBO J 9:2819-2826, 1990.**
Abstract : The c-locus of the mouse is thought to encode tyrosinase, the key enzyme for melanin synthesis in melanocytes of the skin and the eye. Recently, a mouse cDNA was isolated and shown to confer tyrosine activity on a cell line which expressed no specialized functions for melanin synthesis. To verify that the isolated tyrosinase gene is encoded at the genetically well characterized c-locus, a minigene was assembled from tyrosinase cDNA and tyrosinase genomic DNA and used for generation of transgenic mice. Following microinjection of this construct into fertilized eggs of an albino mouse strain, transgenic mice were obtained which showed pigmentation in skin and eyes. By in situ hybridization, we show expression of the transgene in melanocytes of the hairbulb and in the pigmented cell layers of the eye. We conclude that we have rescued the albino mutation (c/c) by introduction and expression of a functional tyrosinase gene.

- **Cohen T, Muller RM, Tomita Y, Shibahara S.**
Nucleotide sequence of the cDNA encoding human tyrosinase-related protein. **Nucleic Acids Res 18:2807-2808, 1990.**

Abstract : A pigment cell-specific cDNA, pMT4, was isolated from a B16 mouse melanoma cDNA library by differential hybridization and it was believed to code for tyrosinase, an essential enzyme of melanin biosynthesis. However, pMT4 was shown to map to the brown (b) locus that dets. the type of melanin produced, which is inconsistent with the assumption that tyrosinase is encoded at the c locus. Subsequently, the protein encoded by pMT4 was shown to possess no tyrosinase activity in transient expression assays and tentatively termed tyrosinase-related protein (TRP), since mouse TRP shares 40% amino acid homol. with the sequence of mouse tyrosinase. Here the nucleotide and deduced amino acid sequence of the cDNA coding for human TRP, a homolog of the mouse b locus gene product is presented. Two cDNA clones were isolated from a cDNA library of S7 human melanoma cells by using the mouse TRP cDNA, pMT4, as a hybridization probe. The assigned reading frame codes for a polypeptide of 527 amino acids with a mol. wt. of 60,000, including a putative signal peptide of 24 amino acids. Human TRP is shorter than mouse TRP by 10 amino acids at the carboxy terminus and the degree of sequence homol. is about 93%.

- **Halaban R, Moellmann G.**
Murine and human b locus pigmentation genes encode a glycoprotein (gp75) with catalase activity. **Proc Natl Acad Sci USA 87:4809-4813, 1990.**
Abstract : Melanogenesis is regulated in large part by tyrosinase (monophenol monooxygenase; monophenol, L-dopa:oxygen oxidoreductase, EC 1.14.18.1), and defective tyrosinase leads to albinism. The mechanisms for other pigmentation determinants (e.g., those operative in tyrosinase-positive albinism and in murine coat-color mutants) are not yet known. One murine pigmentation gene, the brown (b) locus, when mutated leads to a brown (b/b) or hypopigmented (Blt/Blt) coat versus the wild-type black (B/B). We show that the b locus codes for a glycoprotein with the activity of a catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) (catalase B). Only the c locus protein is a tyrosinase. Because peroxides may be by-products of melanogenic activity and hydrogen peroxide in particular is known to destroy melanin precursors and melanin, we conclude that pigmentation is controlled not only by tyrosinase but also by a hydroperoxidase. Our studies indicate that catalase B is identical with gp75, a known human melanosomal glycoprotein; that the b mutation is in a heme-associated domain; and that the Blt mutation renders the protein susceptible to rapid proteolytic degradation.

- **Ivashina TV, Zlotnikov KM.**
Identification of the symbiotic plasmid of Rhizobium phaseoli 693. **Genetika (Moscow) 26:215-221, 1990.**
Abstract : Four cryptic plasmids of R. phaseoli strain 693 were marked by the Tn5-Mob transposon using the suicide PSUP5011 plasmid. The presence of the Mob region in the Tn5 transposon allows the transfer of the marked plasmids to homologous and heterologous recipient strains. Localization of the nodulation and melanin synthesis genes on the non-conjugative pRP693b plasmid of 190 Md mol. mass was shown. The expression of these genes in Agrobacterium tumefaciens and Nod- R. phaseoli strains was demonstrated.

- **Miranda M, Amicarelli F, Bonfigli A, Poma A, Zarivi O, Arcadi A.**
Mutagenicity test for unstable compounds, such as 5,6-dihydroxyindole, using an Escherichia coli HB101/pBR322 transfection system. **Mutagenesis 5:251-255, 1990.**
Abstract : A mutagenicity test for unstable chemical compounds has been devised. The test makes use of (i) in vitro treatment of plasmid pBR322 with the putative mutagen (ii) subsequent transfection of Escherichia coli HB101; (iii) selection either on tetracycline- or ampicillin-containing Eugon agar (iv) cross-antibiotic replica plating and recovery of single antibiotic resistant colonies (v) restriction analysis of pBR322 isolated from single antibiotic resistant colonies. In this work the test has been used to assess the mutagenicity of 5,6-dihydroxyindole, a cytotoxic intermediate of melanin biosynthesis.

7. Tyrosinase and other enzymes

- **Held T, Kutzner HJ.**
Regulation of the tyrosinase biosynthesis in Streptomyces michiganensis (DSM 40 015). **DECHEMA Biotechnol Conf 3:217-220, 1989.**
Abstract : S. michiganensis produces the copper contg., extracellular enzyme tyrosinase, a monooxygenase which is responsible for the formation of the black pigment melanin. Studies of the effect of Cu2+ on

enzyme formation with various methods revealed that the tyrosinase is induced by this metal at the level of transcription. The most efficient induction occurred when Cu2+ was added to the late log phase. Furthermore, tyrosinase and actinomycin, an antibiotic produced by this species, were repressed by ammonia. It could be shown that the repression of tyrosinase depends on the NH4+-concn. and occurs also at the transcription level. Studies with ammonia-derepressed mutants suggested that the repression is mediated by a pleiotrophic intracellular effector.

- **Higa Y, Nakajima K.**
Preparation of kojic acid mono-.gamma.-linolenate as a tyrosinase inhibitor. **Eur Pat Appl, 8 pp., 1989.**
Abstract : The title compd. (I), a melanin formation inhibitor useful for skin-whitening cosmetics, was prepd. by esterification of kojic acid with .gamma.-linolenic acid. AlCl3 was added to a suspension of kojic acid in THF, the mixt. was treated dropwise with linolenyl chloride in CH2Cl2 at 5-10.degree. and stirred 8 h at that temp. to give I. The optical d. of a soln. contg. I, DOPA, and tyrosinase was reduced significantly compared to that of the control.

- **Imokawa G.**
Analysis of carbohydrate properties essential for melanogenesis in tyrosinases of cultured malignant melanoma cells by differential carbohydrate processing inhibition. **J Invest Dermatol 95:39-49, 1990.**
Abstract : In order to clarify the biologic significance of carbohydrate processing in tyrosinases for melanogenesis, we have studied the effect of differential carbohydrate processing inhibitors on the recovery process of interrupted melanization which occurs after exposure of cultured B-16 melanoma cells to the inhibitor of core carbohydrate synthesis, glucosamine (Glc). Treatment of this glycosylation-dependent repigmentation process with the early-stage carbohydrate processing inhibitors deoxynojirimycin (dNM), castanospermine (CS), and monensin (MS) at 0.8 mM, 40 micrograms/ml, and 30 nM, respectively, in the presence of 2 mM theophylline (Tp) almost completely inhibits the reappearance of the pigment 48-72 h after removal of Glc. In contrast, treatment with the later stage carbohydrate processing inhibitor swaisonine (SW) at 40-80 micrograms/ml does not interrupt the repigmentation process. Electrophoretic analysis of tyrosinases in the soluble fractions of these melanoma cells demonstrates that the alteration of soluble tyrosinase isozymes by all the processing inhibitors is associated with a dose-dependent loss of sialic acid-rich T1 tyrosinase and the concomitant appearance or increase of sialic acid-poor tyrosinases. In the large granule fraction, a recovery of membrane-bound tyrosinase (T3) is seen following both MS and SW treatments, whereas dNM treatment results in the substantial loss of T3 tyrosinase. At the electron microscopic level, a translocation of tyrosinase from GERL and coated vesicles to many unmelanized vacuolar premelanosomes occurs in MS-treated cells in contrast to its predominant distribution in the GERL-coated vesicle system of dNM-treated cells, which contain many unmelanized premelanosomes. The present evidence for differential effects on intracellular tyrosinase transfer and melanization by different stages of carbohydrate processing inhibition suggests that asparagine-linked oligosaccharides, relating to the first mannose-trimming stages, determine the function of tyrosinase transfer as well as melanization through a specific intracellular recognition process in pigment cells.

- **Iwata M, Corn T, Iwata S, Everett MA, Fuller BB.**
The relationship between tyrosinase activity and skin color in human foreskins. **J Invest Dermatol 95:9-15, 1990.**
Abstract : Tyrosinase activity was assayed in black and white human foreskin samples by measuring both the hydroxylation of tyrosine to dopa (tyrosine hydroxylase activity) and the conversion of [14C]tyrosine to [14C]melanin (melanin synthesis assay). Enzyme activity was found both in the particulate (75%) and soluble (25%) fractions of the cell. Membrane-bound tyrosinase was readily solubilized by either zwitter-ionic or nonionic detergents. The anionic detergent, sodium cholate, inhibited enzyme activity. Tyrosinase activity in black foreskin homogenates averaged almost three times that in white skin samples (33.8 pmols 3H2O/h/mg skin in black and 12.71 pmoles 3H2O/h/mg skin in white skin), although considerable overlap in activities existed among the two groups. Tyrosinase activities measured with two separate assays, tyrosine hydroxylase and [14C]melanin assays, were similar, suggesting that tyrosine hydroxylase activity is tightly coupled to melanin synthesis. Tyrosinase activity determined by either assay method generally correlated with skin melanin content. Kinetic analysis of tyrosinase from black and white foreskin revealed a Km for tyrosine of 2.5 X 10(-4) M in both skin types. Immunotitration experiments suggested that the difference in tyrosinase

activities between white and black skin may be due, not only to different amounts of enzyme present in the melanocytes, but also possibly to differences in the catalytic activities of the enzyme found in melanocytes of black and white skin.

- **Mishima Y, Oyama Y, Kurimoto M.**
Enzyme formation suppressing carboxylic acids. **Eur Pat Appl, 31 pp., 1989.**
Abstract : Propionic, butyric, and valeric acids and their salts in combination with or without unsatd. fatty acids strong suppressed the formation of tyrosinase, which catalyzes the formation of melanin and thus is a cause of dermatol. troubles. Expts. showed that these acids suppressed tyrosinase formation but did not inhibit tyrosinase. Pharmaceutical formulations were given contg., e.g., Na propionate, Ca butyrate, Na valerate, etc.

- **Mishima Y, Oyama Y, Kurimoto M.**
Enzyme formation suppressing carboxylic acids. **Eur Pat Appl, 31 pp., 1989.**
Abstract : Acetic, lactic and pyruvic acids and their salts in combination with or without unsatd. fatty acids strong suppressed the formation of tyrosinase, which catalyzes the formation of melanin and thus is a cause of dermatol. troubles. Expts. showed that these acids suppressed tyrosinase formation but did not inhibit tyrosinase. Pharmaceuticals and cosmetics were prepd. contg. these acids or salts.

- **Schallreuter KU, Wood JW.**
A possible mechanism of action for azelaic acid in the human epidermis. **Arch Dermatol Res 282:168-171, 1990.**
Abstract : Azelaic acid, and other saturated dicarboxylic acids (C9-C12), are shown to be competitive inhibitors of tyrosinase (KI azelaic acid = 2.73 X 10(-3) M) and of membrane-associated thioredoxin reductase (KI azelaic acid = 1.25 X 10(-5) M). The monomethyl ester of azelaic acid does not inhibit thioredoxin reductase, but it does inhibit tyrosinase, although double the concentration is necessary compared with azelaic acid (KI azelaic acid monomethyl ester = 5.24 X 10(-3) M). Neither azelaic acid nor its monomethyl ester inhibit tyrosinase when catechol is used as a substrate instead of L-tyrosine. Therefore, the weak inhibitory action of azelaic acid on tyrosinase appears to be due to the competition of a single carboxylate group on this inhibitor for the alpha-carboxylate binding site of the L-tyrosine substrate on the enzyme active site. Based on the inhibitor constant on tyrosinase, at least cytotoxic levels of azelaic acid would be required for the direct inhibition of melanin biosynthesis in melanosomes if this mechanism is responsible for depigmentation in the hyperpigmentation disorders lentigo maligna and melasma. Alternatively only 10(-5) M azelaic acid is required to inhibit thioredoxin reductase. This enzyme is shown to regulate tyrosinase through a feedback mechanism involving electron transfer to intracellular thioredoxin, followed by a specific interaction between reduced thioredoxin and tyrosinase. Furthermore, the thioredoxin reductase/thioredoxin system is shown to be a principal electron donor for the ribonucleotide reductases which regulates DNA synthesis.

8. Melanoma

- **Abbott AEJ.**
Melanotic schwannoma of the sympathetic ganglia: pathological and clinical characteristics. **Ann Thorac Surg 49:1006-1008, 1990.**
Abstract : Reports of melanin-producing tumors of Schwann cell origin are extremely rare. Reports of only 9 such tumors arising from sympathetic ganglia have been published previously. Two new cases of melanotic schwannoma of sympathetic ganglia are reported. The pathological and clinical characteristics of these unusual melanotic tumors are discussed.

- **Abe K, Hasegawa H, Kobayashi Y, Fujimura H, Yorifuji S, Bitoh S.**
A gemistocytic astrocytoma demonstrated high intensity on MR images. Protein hydration layer. **Neuroradiology 32:166-167, 1990.**
Abstract : A gemistocytic astrocytoma demonstrating high intensity on both T1W and T2W MR images is reported. Astrocytoma usually shows low density in T1WI. This peculiar astrocytoma showed no hemorrhage,

hemosiderin deposits, melanin or iron. Shortening of T1 relaxation time may be caused by protein hydration layer due to protein rich tumor cells.

- **Ahmed I, Piepkorn MW, Rabkin MS, Meyer LJ, Feldkamp M, Goldgar DE, Skolnick MH, Zone JJ.**
Histopathologic characteristics of dysplastic nevi. Limited association of conventional histologic criteria with melanoma risk group. **J Am Acad Dermatol 22:727-733, 1990.**
Abstract : Studies of dysplastic melanocytic nevi (DMN) have suggested that lesions from patients with a personal or family history of malignant melanoma are histologically more atypical than are those from control populations without such histories. To evaluate this possibility, we examined histologic sections of DMN that had been removed from patients in three groups. Group A consisted of 17 subjects with a past history of melanoma; group B comprised 79 subjects with DMN and a family history of melanoma in first-degree relatives; group C consisted of 64 subjects who were unrelated spouses of members of groups A and B. For each group, sections of DMN were initially selected on the basis of architectural atypia as defined by the National Institutes of Health Consensus Conference. All biopsy material was then further evaluated for four histologic features suggested to be discriminatory for DMN associated with increased melanoma risk; the features are degree of junctional activity, irregularity of melanocytic nests, presence of dusty melanin, and size of melanocytic nuclei. A subjective rating was given for each on a 0 to 3 scale of increasing severity. Three pathologists individually rated the specimens without knowledge of the patient group. The means of the individual observer cumulative scores of the four histologic criteria for each biopsy specimen and the average of these means from the three observers exhibited a trend to increasing values from groups C to A, but none of the differences reached statistical significance.

- **Aldred MJ, Gray AR.**
A pigmented adenomatoid odontogenic tumor. **Oral Surg Oral Med Oral Pathol 70:86-89, 1990.**
Abstract : Occasional reports have described the presence of melanin in various odontogenic lesions. A case of melanin pigmentation in an adenomatoid odontogenic tumor is described.

- **Alena F, Jimbow K, Ito S.**
Melanocytotoxicity and antimelanoma effects of phenolic amine compounds in mice in vivo. **Cancer Res 50:3743-3747, 1990.**
Abstract : A phenolic amine compound, 4-S-cysteaminylphenol (4-S-CAP), is a potent depigmenting agent. To develop more efficacious antimelanoma agents, we synthesized four homologues of 4-S-CAP: N-acetyl-4-S-CAP (N-Ac-4-S-CAP), alpha-methyl-4-S-CAP, 4-S-homo-CAP, and N,N'-dimethyl-4-S-CAP. We tested these five compounds in mice in vivo. After s.c. or i.p. injection of saline solution (in control groups) or one of the compounds, follicular melanocytes were examined by light and electron microscopy to assess the degree of melanocytotoxicity; N-Ac-4-S-CAP induced the most depigmentation (98%), whether given i.p. or s.c. After injection of 4-S-CAP or N-Ac-4-S-CAP, the number of murine B16F10 melanoma colonies formed in the lungs was determined; 4-S-CAP and N-Ac-4-S-CAP were almost equally effective, reducing the colonies to 32 and 25% of mean control, respectively. Metabolic studies of the urine showed 9% of 4-S-CAP and 20% of N-Ac-4-S-CAP injected i.p. were excreted unchanged in 24 h; 1.3% of the N-Ac-4-S-CAP was excreted as 4-S-CAP, indicating some conversion. We conclude that N-Ac-4-S-CAP is a suitable model for developing chemotherapy to treat melanoma characterized by high tyrosinase activity and melanin synthesis.

- **Anichini A, Mortarini R, Berti E, Parmiani G.**
Multiple VLA antigens on a subset of melanoma clones. **Hum Immunol 28:119-122, 1990.**
Abstract : A panel of 19 melanoma clones was characterized by fluorescence activated cell sorter analysis for the expression of distinct integrin receptors in the very late activation antibody subfamily. A group of five melanoma clones (2/4, 2/14, 2/17, 2/51, and 2/60) was identified that shared coordinate expression of multiple VLA antigens. These same clones were more susceptible, in terms of lytic units, to cell-mediated lysis and also expressed the lower melanin content per cell in the panel. The data suggest that in a heterogeneous neoplastic population, distinct features such as susceptibility to cell-mediated lysis and expression of receptors for extracellular components are variables preferentially associated with cells in an early stage of differentiation.

- **Atlas SW, Braffman BH, LoBrutto R, Elder DE, Herlyn D.**
Human malignant melanomas with varying degrees of melanin content in nude mice: MR imaging, histopathology, and electron paramagnetic resonance. **J Comput Assist Tomogr 14:547-554, 1990.**
Abstract : The etiology of the paramagnetic relaxation enhancement seen in malignant melanoma on proton magnetic resonance (MR) images has been the subject of many recent investigations and has been ascribed to iron from associated hemorrhage or chelated metal ions, rather than directly due to melanin. The purpose of this study was to correlate proton relaxation times on MR images in malignant melanomas with histopathologic features (i.e., degree of pigmentation, iron deposition, and necrosis), water content, and electron paramagnetic resonance (EPR) spectra to elucidate the etiology of the relaxation behavior demonstrated by these neoplasms. Cultured cells derived from human malignant melanoma metastases were implanted subcutaneously into nude mice. Twelve separate lesions were evaluated in 10 mice. Magnetic resonance imaging was performed in vivo at 1.9 T using spin echo and inversion recovery acquisitions for the purposes of calculating T1, T2, and proton density [N(H)]. Histopathologic examination was performed on specimens resected immediately after imaging, using hematoxylin/eosin, Prussian blue, and Fontana stains to assess tumor necrosis, and iron and melanin content. Dry/wet weight ratios and EPR spectra were also obtained on resected specimens. Our results indicate that T1 shortening correlates with increasing melanin content and not with increasing iron deposition, EPR-active metallic cations, necrosis, or water content. In fact, a presumably unrelated statistical correlation was found between increased iron and T1 prolongation. The T2 relaxation times did not correlate with the presence of any single factor other than proton density. Although the unique relaxation behavior of nonhemorrhagic malignant melanoma in vivo cannot be traced to a single cause, our data suggest that, contrary to previous investigations, it is strongly influenced by the presence of melanin rather than iron or other naturally occurring paramagnetic ions.

- **Borazjani G, Prem KA, Okagaki T, Twiggs LB, Adcock LL.**
Primary malignant melanoma of the vagina: a clinicopathological analysis of 10 cases. **Gynecol Oncol 37:264-267, 1990.**
Abstract : We retrospectively analyzed clinicopathological findings in 10 cases of primary malignant melanoma of the vagina. The main presenting symptoms were vaginal bleeding, vaginal discharge, and feeling of a mass. The tumors were predominantly located in the lowest one-third and in the anterolateral aspect of the vagina. Patients underwent various surgical procedures, radiation therapy, and chemotherapeutic modalities. The mean survival time and the recurrence time from the time of diagnosis were 15 and 8 months, respectively. The tumors were examined for histological characteristics of cell type, presence of melanin pigment, depth of invasion, vascular invasion, intraepithelial spread, junctional activity, and mitotic count. Of all these histological variables, the mean survival time had a significant correlation to mitotic count (P less than 0.04). We concluded that patients with lower mitotic counts (less than 6 per 10 HPF) had better survival (21 months) compared to patients with mitotic counts greater than 6 per 10 HPF who had a mean survival of only 7 months.

- **De Pauw-Gillet MC, Siwek B, Pozzi G, Sabbioni E, Bassleer RJB.**
Control of B16 melanoma cells differentiation and proliferation by copper(II) sulfate and vitamin C. **Anticancer Res 10:391-395, 1990.**
Abstract : The effects exerted by CuSO4, in the presence or absence of vitamin C, on melanogenesis and proliferation in mouse B16 melanoma cells in culture were analyzed either in serum-free (MEM-N2) or in serum-supplemented media. The stimulation or the inhibition of these cellular parameters can be induced, depending on the metal concn., the presence or absence of vitamin C, the compn. of the culture medium and on the type of culture (subconfluent or clonal). Vitamin C toxicity for B16 cells was generally increased in serum-free medium, in clonal cultures, or in the presence of CuSO4.

- **De Pauw-Gillet MC, Siwek B, Pozzi G, Sabbioni E, Bassleer RJ.**
Effects of FeSO4 on B16 melanoma cells differentiation and proliferation. **Anticancer Res 10:1029-1033, 1990.**
Abstract : The effects exerted by FeSO4, in the presence or absence of vitamin C, on melanogenesis and proliferation in mouse B16 melanoma cells in culture were analysed either in serum-free (MEM-N2) or in serum-supplemented media. These cellular parameters can be either stimulated or on the contrary inhibited, depending on the metal concentration, the presence or the absence of vitamin C and serum, and on the type

of culture (subconfluent or clonal). Vitamin C toxicity for B16 cells was decreased in the presence of FeSO4.

- **Garcia-Bragado F, Cabello A, Guarch R.**
Melanotic medulloblastoma. Ultrastructural and histochemical study of a case. **Arch Neurobiol (Madr) 53:8-12, 1990.**
Abstract : A electron microscopic and immunohistochemical study of a Melanotic medulloblastoma is reported. The cerebellar tumor was located in the vermis of a 6-year-old boy, dead 11 months after diagnosis. The tumor consisted of medulloblastoma-like areas with focal differentiation and pseudoepithelial structures pigmented with melanin. Electron microscopy showed melanosomes and tight junctions in pigmented areas. On immunohistochemistry, the cytoplasm of melanotic cells were positive to S-100 protein and the differentiated glial cells to GFAP. The tumor histogenesis, its relationship with other pigmented tumors of the CNS and their low frequency is commented on.

- **Hammersmith SM, Terk MR, Jeffrey PB, Connolly SG, Colletti PM.**
Magnetic resonance imaging of nasopharyngeal and paranasal sinus melanoma. **Magn Reson Imaging 8:245-253, 1990.**
Abstract : Malignant melanoma of the nasopharynx and paranasal sinuses is relatively rare. We retrospectively reviewed the magnetic resonance appearance of five cases and correlated this with the histopathological appearance. In all cases, the magnetic resonance (MR) images clearly demonstrated the precise anatomic extent of the tumor and were sensitive in assessing intracranial extension and invasion into surrounding structures, including the skull base. Three cases were reviewed for histopathological evidence of melanin, hemosiderin, and acute hemorrhage. One case was reviewed for melanin and hemorrhage only. The findings in this series suggest that melanoma of the nasopharynx and paranasal sinuses have extremely variable amounts of paramagnetic substances, both melanin and products of hemorrhage. T1 shortening appears to be more often a reflection of the paramagnetic effects associated with products of hemorrhage rather than the presence of melanin.

- **Hashimoto S, Ishibashi J, Inokari S.**
A case of amelanotic melanoma originating in the adrenal medulla. **Nippon Naika Gakkai Zasshi 79:678-679, 1990.**

- **Hill SE, Bleehen SS, MacNeil S.**
I 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 I alpha-25-dihydroxyvitamin D3 (I alpha-25-(OH)2D3) have been reported to play a role in melanogenesis. Physiological concentrations of I alpha-25-(OH)2D3 were found to acutely elevate intracellular free calcium (using Fura 2) in B16 primary (Io) cells. Membrane phosphoinositide turnover was unaffected by I alpha-25-(OH)2D3. The rise in intracellular free calcium was entirely dependent on extracellular calcium and was not mimicked by vitamin D3. However, in neither B16-Io nor B16-F1 melanoma cells did vitamin D3 or I alpha-25-(OH)2D3 increase melanin production.

- **Hill SE, Rees RC, MacNeil S.**
The regulation of cyclic AMP production and the role of cyclic AMP in B16 melanoma cells of differing metastatic potential. **Clin Exp Metastasis 8:475-489, 1990.**
Abstract : The nature of the relationship between agonist-stimulated cyclic AMP production and metastatic potential was examined in detail for four B16 melanoma cell lines of varying metastatic potential. Highly metastatic cells (B16 F10C1) appeared to differ from cells of low metastatic potential (B16 F1C29) in the degree to which cyclic AMP production in intact cells was stimulated by protein kinase C activation. No significant difference was found in the adenylate-cyclase enzyme activities of the broken cells, irrespective of the agonist used, or in the distribution of cyclic AMP between the intracellular and extracellular compartment. Although B16F1, F10 and F10C1 cells all produced equally pigmented tumors in vivo, the cells differed in their melanogenic response to cyclic AMP elevating agents in vitro: the least metastatic cells produced least agonist-induced cyclic AMP but this induced greatest tyrosinase activation and melanin production in vitro; conversely, the more metastatic cells produced more cyclic AMP but less tyrosinase activation and melanin production in response to agonist stimulation. Thus, agonist-stimulated cyclic AMP

production does not appear to be coupled to the differentiated function of melanogenesis for highly metastatic B16 melanoma cells.

- **Kiguchi K, Constantinou AI, Huberman E.**
Genistein-induced cell differentiation and protein-linked DNA strand breakage in human melanoma cells. **Cancer Commun 2:271-277, 1990.**
Abstract : Genistein, an in vitro inhibitor of topoisomerase II and tyrosine kinases, elicited an inhibition of growth and increased melanin content in five human melanoma cell lines, after six days of treatment at a concentration of 45 microM. In two lines examined more thoroughly, HO and SK-MEL-131, treatment with genistein also increased other markers of differentiation, including tyrosinase activity, reactivity with CF21 monoclonal antibody, and dendrite-like structure formation. The genistein-evoked increases in melanin content and tyrosinase activity were concentration- and time-dependent. Treatment of HO and SK-MEL-131 cells with 45 microM genistein for 24 hr or 60-600 microM genistein for only 1 hr resulted in an increase in protein-linked DNA strand breaks. Our results suggest an association between the genistein-evoked, protein-linked, DNA strand breaks and the genistein-induced differentiation of human melanoma cells.

- **Maiorana A, Bagni A, Sannicola C, Barca F.**
Clear cell sarcoma of the hand. Description of a case. **Pathologica 82:95-100, 1990.**
Abstract : Clear cell sarcoma of the hand. Case report. Histologic, ultrastructural and immunohistochemical features of a case of clear cell sarcoma (also called malignant melanoma of soft parts) arisen in the index finger of the right hand in a 28 year-old woman are described in this report. Typical forms of this tumor are deeply located and associated with tendons and aponeuroses, lacking cutaneous involvement. The tumor has to be differentiated from other benign and malignant lesions of the soft parts, such as a giant cell tumor of tendon sheaths and a fibrosarcoma. The demonstration of melanin and a positive immunohistochemical reaction for S-100 protein and HMB-45 (a melanin-associated antigen) can assist in the differential diagnosis. In spite of a slow and protracted clinical course, many of the patients experience multiple local recurrences and distant metastases. Prognosis is poor in a high percentage of cases.

- **Mariani M, Supino R.**
Morphological alterations induced by doxorubicin in B16 melanoma cells. **Cancer Lett 51:209-212, 1990.**
Abstract : In B16 melanoma, cells morphologically different can be distinguished. In order to establish possible correlations between cell morphology and drug-response, the cytotoxic response to doxorubicin was analyzed. The two subpopulations, represented by two types of colonies, showed a different degree of sensitivity to doxorubicin. Moreover, following treatment, colonies strongly altered in their morphology were found, suggesting a differentiating activity of doxorubicin (dendritic prolongations, increase of intracellular melanin, block of cell proliferation). These results suggest that doxorubicin, besides having a different cytotoxic effect of the two cell subpopulations, induces in this cell line morphological alterations consistent with a differentiation process.

- **Mi C.**
Clear cell sarcoma: a clinicopathological study of 11 cases. **Chung-hua Ping Li Hsueh Tsa Chih 18:221-223, 1989.**
Abstract : The clinicopathologic, ultrastructural and immunohistochemical features of eleven cases of clear cell sarcoma are described. There were 6 males and 5 females with an average age of thirty-six (10-59 years). Tumors were found arising from the tendons, aponeuroses and fascial structures with a predilection for the lower and upper extremities. Follow-up data was available in 8 patients. Five of them are alive. Nevertheless, 3 of the five showed evidence of recurrence or metastasis. The other 3 patients died of tumor with metastasis. Microscopically, the tumors were composed of short fascicles of fusiform cells with a clear to eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli. Melanin was demonstrated in 5 cases and S-100 was known present focally in all cases, but no positive keratin staining was obtained. Electron microscopic studies revealed cell attachments and mature melanosomes. The exact histogenesis remains obscure, but our ultrastructural and immunohistochemical findings support the idea of neural crest origin of this tumor.

- **Nagy P, Csaba I, Kadas I.**
Malignant melanoma metastatic to the endometrium. Cytologic findings in a direct endometrial sample. **Acta**

Cytol 34:382-384, 1990.

Abstract : A 38-year-old woman was hospitalized with uterine bleeding and a history of malignant melanoma. Endometrial biopsy had already documented the presence of rare uterine metastases from a cutaneous lesion surgically removed from the back 1.5 years previously. While a cervicovaginal smear was negative, smears of a sample obtained from the uterine cavity by the Mi-Mark endometrial helix showed the cytomorphologic features of melanoma, corresponding to those seen in the primary lesion. The finely granular cytoplasmic pigment seen in the smears was proven to be melanin by the use of potassium permanganate oxidation. The patient then underwent hysterectomy. This case demonstrates the potential utility of direct endometrial sampling in diagnosing difficult cases.

- **Nakabeppu Y, Nakajo M, Iwashita S, Tanoue T, Shinohara S.**
[123I-N-isopropyl-p-iodoamphetamine scintigraphy in patients with malignant melanoma. **Radioisotopes 39:163-167, 1990.**

Abstract : N-Isopropyl-p-[123I]iodoamphetamine (123I-IMP) has been widely used in patients with cerebral vascular damage and epilepsy. The potential of 123I-IMP accumulation in the melanin producing cells has been reported since early stage of its development. Some authors reported the accumulation of 123I-IMP in the malignant melanoma of human and animals. We evaluated the 123I-IMP scintigraphy in 9 patients with malignant melanoma. Patients were classified into two groups: A, 4 patients with 8 lesions; B, 5 post-operative patients without lesions. In group A, the 123I-IMP uptake was seen in 4 of 8 lesions. The smallest true positive lesion was located at skin and its size was 15 mm in diameter. Two of the visualized 4 lesions were amelanotic malignant melanomas. This fact suggests that uptake of 123I-IMP in malignant melanoma may be related to the processes of melanin synthesis. In group B, two abnormal deposits had been seen in the right thigh of a female patient. However no abnormality was seen in the following 67Ga scintigraphy, TCT, MRI, and repeated 123I-IMP scintigraphy. Therefore the abnormal deposits were considered to be the false positive lesions due to urinary contamination.

- **Nakamura T, Matsuno M, Kageshita T, Arao T.**
Expression of HLA-class II antigens in malignant melanoma. **Nippon Hifuka Gakkai Zasshi 100:49-56, 1990.**

Abstract : Sixteen primary and ten metastatic melanoma lesions were stained in indirect immunoperoxidase with HLA-DR, DP and DQ monoclonal antibodies. In primary lesions, HLA-DR, DQ and DP antigens were much more expressed in nodular melanoma lesions than in acral lentiginous melanoma lesions. In metastatic lesions HLA-DR, DQ and DP antigens were expressed more than in primary lesions, but there were no significant differences between ALM and NM. In primary melanoma, 82%, 75%, and 75% of lesions tested were stained by anti HLA-DR, DP and DQ monoclonal antibodies, respectively. In metastatic melanoma, 90% of the lesions tested were stained by anti HLA-DR, DP and DQ monoclonal antibodies. HLA-class II antigens on melanoma cells were much more expressed in cases of high levels of invasion, high degrees of lymphocyte infiltration, and lower melanin content.

- **Nakhleh RE, Wick MR, Rocamora A, Swanson PE, Dehner LP.**
Morphologic diversity in malignant melanomas. **Am J Clin Pathol 93:731-740, 1990.**

Abstract : A review was conducted of 335 malignant melanomas to identify variant morphologic patterns that might be confused with other tumors. In all, 27 predominantly amelanotic neoplasms with unusual histologic features were selected for additional study. These included nine with an adenoid or pseudopapillary pattern, seven small cell neoplasms, five with prominent myxoid stroma, four with a hemangiopericytoma-like appearance, and two composed of neoplastic cells with a signet-ring configuration. A diagnosis of melanoma was confirmed in all cases by Fontana-Masson strains for melanin pigment, electron microscopic examination, or the results of immunohistochemical analyses for cytokeratin, vimentin, S-100 protein, and the HMB-45 antigen. One tumor was associated with a congenital hairy melanocytic nevus, five were vulvovaginal lesions, four arose in the sinonasal tract, and one occurred in the rectum. Four of the specified microscopic patterns were observed in both primary and secondary neoplasms; the two signet-ring cell melanomas were recurrent lesions. The authors conclude that malignant melanomas may assume the histologic guise of adenocarcinomas, small cell carcinomas, and sarcomas, in a variety of tissue sites. Special studies designed to detect melanocytic differentiation are therefore appropriate in diverse differential diagnostic settings.

- **Nigro MA, Castellani L, Chieregato C.**
Etiopathogenesis of malignant melanoma of the skin. III. Disease factors inherent in the environment. Pathogenetic hypothesis. **G Ital Dermatol Venereol 125:117-123, 1990.**
Abstract : Sunlight, particularly its UVB component, is thought to be the most important environmental factor for oncogenesis of melanoma. Its intensity, at the ground level, is a positive function of altitude and a negative function of latitude. Sun exposure and susceptibility in childhood seem to be major risk factors at least in Anglo-saxon countries. UV radiations are able to act as complete carcinogen. Eumelanin/pheomelanin ratio also appears as an important risk factor. Ionizing radiations, heat and traumas have been seldom related to melanoma carcinogenesis. Several chemicals, among them drugs and toxic drugs, add to the list of possible causative agents. Loss of alleles encoding for suppressor factors, caused by UV radiation, might play a significant role in carcinogenesis. A model is proposed, for "mediterranean" vs "caledonian" melanoma, in which the phenotypic sequence melanocytic nevus--melanoma would exhibit peculiar characteristics.

- **Rofstad EK, Zaffaroni N, Hystad ME.**
Heterogeneous radiation and heat sensitivity in vitro of human melanoma xenograft lines established from different lesions in the same patient. Comparisons with the radiation and heat sensitivity of cells isolated from the donor patient's surgical specimens. **Int J Radiat Biol 57:1113-1122, 1990.**
Abstract : Human melanoma xenograft lines were established in athymic nude mice (BALB/c-nu/nu/BOM) from the primary tumour (OKL-PRI), a s.c. metastasis (OKL-SCM) and a lymph node metastasis (OKL-LNM) in the same patient. The three lines differed in growth rate, melanin content, and radiation and heat sensitivity in vitro. The OKL-PRI line grew more slowly than the OKL-SCM and OKL-LNM lines and was the only line that synthesized significant amounts of melanin. The D0 values were 0.96 +/- 0.07 Gy, 0.87 +/- 0.07 Gy and 1.52 +/- 0.09 Gy (X-rays); 143 +/- 21 min, 109 +/- 12 min and 195 +/- 40 min (heat, 42.5 degrees C); and 21.3 +/- 2.7 min, 15.3 +/- 1.7 min and 26.7 +/- 3.0 min (heat, 44.5 degrees C) for the OKL-PRI, OKL-SCM and OKL-LNM line, respectively. The ranking of the lines in treatment sensitivity was equal for radiation and heat. The radiation and heat sensitivities were similar to those for cells isolated directly from the surgical specimens of the donor patient. The lines were thus established from a single neoplastic disease without artificial cloning in vitro or in vivo, and the cellular radiation and heat sensitivity did not change during the establishment procedure, suggesting that they constitute a relevant experimental model system for studies of clonal tumour heterogeneity in response to radiation and hyperthermia treatments.

- **Rosenblum MK, Erlandson RA, Aleksic SN, Budzilovich GN.**
Melanotic ependymoma and subependymoma. **Am J Surg Pathol 14:729-736, 1990.**
Abstract : We report two examples of melanin production by human gliomas. One was a grossly pigmented, well-differentiated ependymoma resected from the left frontoparietal region of a 13-year-old girl. The patient received radiotherapy and was free of tumor 12 years after operation. The second example was a pigmented subependymoma incidentally discovered at the autopsy of a 52-year-old man. Neoplastic cells containing an intracytoplasmic pigment satisfying histochemical criteria for melanin were present in both cases. Electron microscopic study of the melanotic ependymoma revealed electron-dense granules in the cytoplasm of cells forming rosettes. Premelanosomes were not detected. While the mechanism of melanogenesis in these cases is obscure, they support the potential of glial derivatives to produce melanin and indicate that melanogenesis in such neoplasms may have no adverse prognostic import.

- **Terzakis JA, Opher E, Melamed J, Santagada E, Sloan D.**
Pigmented melanocytic schwannoma of the uterine cervix. **Ultrastruct Pathol 14:357-366, 1990.**
Abstract : A 47-year-old woman had a lesion of the uterine cervix that presented clinically as a protruding or aborted leiomyoma. Grossly the tumor occupied a substantial portion of the cervical and endocervical region. Histologically it showed a spindle cell neoplasm arranged in large fascicles that penetrated deeply into the fibromuscular wall of the cervix. The tumor cells had abundant pink cytoplasm that contained considerable brown melanin granules confirmed by Fontana's stain. Cytologically nuclear pleomorphism, hyperchromatism, and giant nuclear forms were observed. Mitoses were also seen. Localized nuclear palisading was present. Electron microscopic examination of paraffin-embedded material revealed numerous premelanosomes and opaque granules that were compatible with mature melanosomes, thus confirming melanogenesis in the tumor. Tumor cells exhibited focal projections, and the connective tissue showed

abnormal spacing of collagen. Basal lamina material was noted focally on tumor cell surfaces. Immunocytochemistry showed a positive reaction to S-100 protein and HMB-45 in tumor cell cytoplasm.

- **Umemura T, Naoi M, Takahashi T, Fukui Y, Yasue T, Ohashi M, Nagatsu T.**
Cytotoxic effect of 1-methyl-4-phenylpyridinium ion on human melanoma cell lines, HMV-II and SK-MEL-44, is dependent on the melanin contents and caused by inhibition of mitochondrial electron transport. **Biochem Med Metab Biol 44:51-58, 1990.**
Abstract : MPP+, an oxidative metabolite of a neurotoxin, MPTP, was found to be cytotoxic to human melanoma cell lines, HMV-II and SK-MEL-44. After 3 days of culture in the presence of MPP+, a larger amount of MPP+ was accumulated in HMV-II cells than in SK-MEL-44 cells, which correlated well with the melanin contents; HMV-II cells contain larger amounts of melanin than SK-MEL-44 cells. After 6 days of culture in the presence of MPP+, the cytotoxicity of MPP+ on these cell types was evaluated by counting cell numbers with the dye exclusion test and double-layer soft agar clonogenic assay. It was found that exposure to MPP+ reduced the survival of HMV-II cells more significantly than that of SK-MEL-44 cells. In HMV-II cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay was used to elucidate the mechanism of MPP+ lethality. The formazan formation was reduced markedly by the presence of MPP+ at concentrations much lower than those required for cell death. These results suggest that cytotoxicity of MPP+ may be ascribed to its accumulation due to high affinity for melanin, and to inhibition of the enzymes utilizing ubiquinone in the mitochondrial respiratory chain.

- **Uozumi A, Saegusa T, Ohsato K, Yamaura A.**
Computed tomography and magnetic resonance imaging of nonhemorrhagic, metastatic melanoma of the brain. Case report. **Neurol Med Chir (Tokyo) 30:143-146, 1990.**
Abstract : Malignant melanoma frequently metastasizes to the central nervous system. Characteristic features of such lesions are increased density on computed tomography (CT) and shortening of the T1 and T2 on magnetic resonance (MR) imaging. Intratumoral hemorrhage, rather than melanin, is considered to be mainly responsible for these unique radiological features. The authors report a case involving a nonhemorrhagic, metastatic intracerebral melanoma. CT revealed a high-density mass, and MR imaging demonstrated a hyperintense mass both on T1- and T2-weighted images. These observations suggest that melanin is in fact a major determinant of the increased density on CT scans and the high signal intensity on T1-weighted MR images. On T2-weighted images, the paramagnetic effect appears to vary according to the melanin content.

- **Warter A, George-Diolombi G, Chazal M, Ango A.**
Melanin in a dentigerous cyst and associated adenomatoid odontogenic tumor. **Cancer 66:786-788, 1990.**
Abstract : The authors report on a case of dentigerous cyst associated with odontogenic adenomatoid tumor in an 8-year-old black Nigerian boy. Both the cyst and the tumor contained melanocytes and melanin-laden epithelial cells. To their knowledge this is the first reported case of melanotic follicular cyst and adenomatoid tumor. A review of the literature revealed that melanin is rarely found in odontogenic lesions. Since the neural crest influence on the development of odontogenic tissues is well established, the occurrence of melanocytes in these tissues is not surprising. A racial predisposition may be present; black patients predominated in the 15 reported cases of melanotic odontogenic lesions.

9. Eye

- **Coscas G, Soubrane G, Sterkers M, Glacet-Bernard A.**
The dye laser: experimental and clinical results in subretinal macular neovascularization]. **J Fr Ophtalmol 12:613-621, 1989.**
Abstract : The tunable dye laser provides now a large assortment of monochromatic wavelengths that allow selective targeting on individual tissues in the retina, according to in vitro studies showing extinction coefficient of ocular pigments (xanthophyllic pigment, rhodopsin, melanin, lipofuschin, hemoglobin) for different laser wavelengths. Confluent heavy and juxtafoveolar laser burns in eyes of cynomolgus monkeys showed, after 38 days, similar lesions in all the wavelength studied (green, red, yellow, orange) at the level of the choriocapillaris, Bruch's membrane, pigment epithelium and photoreceptor's layers. However, the inner retina layers were discretely more damaged with yellow and orange than with green and red. In clinical

approach, the effects of these different wavelength haven been compared in human eyes presenting with subfoveol new vessels and disciform age-related macular degeneration. After healing, the scar was similar biomicroscopically, and on fluorescein angiography with all wavelengths studied (green, red, yellow, orange). In conclusion, dye laser seems to be efficient and easily tunable for photocoagulation in macular area.

- **Damato BE, Foulds WS.**
Tumour-associated retinal pigment epitheliopathy. **Eye 4:382-387, 1990.**
Abstract : Choroidal tumours are associated with several degenerative changes in the overlying tissues, which can be called 'Tumour-Associated Retinal Pigment Epitheliopathy (TARPE)'. These changes include (i) proliferation, detachment, atrophy, and metaplasia of the retinal pigment epithelium, (ii) the accumulation of hard and soft drusen and basal laminar deposits in Bruch's membrane, (iii) disorganisation of the choriocapillaris, (iv) atrophy, cystic degeneration and detachment of the retina. The changes at the chorio-retinal interface are clinically relevant because they can exacerbate visual loss. In addition, they can be misinterpreted on ophthalmoscopy and fluorescein angiography. An amelanotic choroidal tumour may appear to be pigmented on ophthalmoscopy because of lipofuscin and melanin accumulation overlying the tumour. The hyperfluorescence associated with pigmented choroidal melanomas is more likely to be related to degenerative changes in the retinal pigment epithelium than to dye leakage from abnormal tumour vessels.

- **Kitagawa K.**
Autofluorescence of ocular fundus for the evaluation of retinal aging changes. **Aichi Ika Daigaku Igakkai Zasshi 18:43-55, 1990.**
Abstract : Age-related autofluorescence changes were studied by fluorophotometry at the macular region of ocular fundus from 3 mo-72-yr-old humans. Autofluorescence at the maculae of aphakic eyes increased with age. Pigment granules emitting yellow autofluorescence were identified only in the retinal pigment epithelium by fluorescence microscopy using filter combinations of either 366 nm for excitation with 420 nm for barrier, or 490 nm for excitation with 515 nm for barrier. The peaks of the emission spectrum of the autofluorescence were about 460 nm for above mentioned 2 filter combinations by microphotometry. Electron microscopic examn. of the retinal pigment epithelia revealed that melanin granules decreased with age in no., while melanolipofuscin and lipofuscin granules increased. Thus, the ocular fundus autofluorescence mainly originates from lipofuscin in human retinal pigment epithelium, and the increase of autofluorescence with age is attributed to the accumulation of lipofuscin.

- **Kiuchi Y, Mishima HK, Nagata A, Kurokawa T, Ishibashi S.**
Ocular hypotensive effect of griseolic acid-ester, a cAMP phosphodiesterase inhibitor, in pigmented and albino rabbits. **Atarashii Ganka 7:410-414, 1990.**
Abstract : The ocular hypotensive effect of griseolic acid-ester(Ga-ester), a cAMP phosphodiesterase inhibitor, and the binding ability of GA-ester to synthetic melanin by spectrophotometry were examd. Topical application of 2% GA-ester significantly decreased intraocular pressure(IOP) in albino rabbits. However, in pigmented rabbits instillation of GA-ester had little effect on IOP. Subconjunctival injection of GA-ester reduced IOP significantly in both rabbits. The hypotensive effect was lower in pigmented rabbits than in albino rabbits. GA-ester was bound to synthetic melanin by a concn.-dependent manner. Hence, melanin in ocular tissues may alter the ocular hypotensive effect of GA-ester.

- **Lauritzen K, Augsburger JJ, Timmes J.**
Vitreous seeding associated with melanocytoma of the optic disc. **Retina 10:60-62, 1990.**
Abstract : The authors describe an unusual case of melanocytoma of the optic disc associated with seeding of extracellular pigment or melanin-containing macrophages into the overlying vitreous. The possible mechanisms responsible for this clinical picture are discussed.

- **Lloyd WC 3d, Eagle RCJ.**
Congenital hypertrophy of the retinal pigment epithelium. Electron microscopic and morphometric observations. **Ophthalmology 97:1052-1060, 1990.**
Abstract : Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is a well-circumscribed, flat, pigmented fundus lesion that is stable and generally nonprogressive. Light and electron microscopy and morphometric analysis was used to study a lesion with the clinical characteristics of CHRPE found in an eye

enucleated for a posterior segment malignant melanoma. These studies showed that the lesion was composed of tall, maximally pigmented RPE cells that had a density 1.7 times greater than the density of the adjacent normal peripheral RPE. These observations suggested that cellular hyperplasia and hypertrophy may contribute to CHRPE. Ultraviolet fluorescence microscopy showed no autofluorescent granules of lipofuscin in the CHRPE, suggesting that the lesion's constituent cells lack the capacity to phagocytose and digest photoreceptor outer segments. Photoreceptor degeneration in the overlying retina consequent to this functional defect could be responsible for the localized visual field defects that typically occur in patients with CHRPE.

- **Mosteller MW, Margo CE.**
Visual loss from endothelial cell melanization of the cornea. **Am J Ophthalmol 109:733-735, 1990.**

- **Opas M, Dziak E.**
Effects of a tumor promoter, 12-O-tetradecanoylphorbol 13-acetate (TPA), on expression of differentiated phenotype in the chick retinal pigmented epithelial cells and on their interactions with the native basement membrane and with artificial substrata. **Differentiation (Berlin) 43:20-28, 1990.**
Abstract : Chick retinal pigmented epithelial (RPE) cells on Engelbreth-Holm-Swarm tumor (BM-matrigel) were treated with TPA to promote the transformed phenotype and diminish cell traction. In contrast to most cell types TPA treatment induced RPE cells to increase their spread area. TPA promoted RPE cell spreading on BM-matrigel and changed the spatial organization of actin and actin-assocd. proteins in the cytoskeleton-extracellular matrix linkage complexes, uncoupling actin from its extracellular counterpart. TPA did not affect other components of the cytoskeleton in RPE cells. TPA also affected labile adhesions i.e., focal contacts and adherens junctions in statu nascendi, but preformed, stable adherens junctions were resistant to TPA. TPA enhanced proliferation, blocked melanogenesis, and thus inhibited differentiation of RPE cells grown on either artificial substrata or their natural basement membrane.

- **Sai S, Usukura J.**
Structure and function of the retinal pigment epithelium. **Saibo 22:144-147, 1990.**
Abstract : A review, with 17 refs., on structure and function of retinal pigment epithelium; the phagocytic activity, energy metab., vitamin A metab., melanin granule in photoadaptation, blood-retina barrier function, and role of cytoskeleton in the retinal pigment epithelium function are discussed.

- **Yamashita H, Yamamoto T.**
Changes in distribution of chloride ions in embryonic chicken retinal pigment epithelium. **Jpn J Ophthalmol 34:22-29, 1990.**
Abstract : Changes in the distribution pattern of chloride ions were studied in the embryonic chicken retinal pigment epithelium (RPE), chloride ions being deposited as silver chloride (AgCl) in the RPE and observed by electron microscope. Before the 7th day after fertilization, the chloride ions did not accumulate anywhere in the tissue. From the 8th day, the chloride ions accumulated in the cytoplasm and the intercellular space, first in the posterior fundus RPE and later in the anterior fundus RPE. Melanin pigment granules and apical microvilli of the RPE developed from the posterior fundus toward the anterior fundus in parallel with the changes in the distribution pattern of chloride ions. These results suggest that the chloride ion transport function of the RPE appears first in the posterior fundus on the 8th day after fertilization and thereafter the development of the chloride ion transport moves to the anterior fundus. This development of the transport function appears to progress in parallel with the morphological development of the RPE cells.

10. Other

- **Fukuda M, Sasaki K.**
Changes in the antibacterial activity of melanin-bound drugs. **Ophthalmic Res 22:123-127, 1990.**
Abstract : Affinity to melanin and changes of antibacterial activity of melanin-bound drugs were examined in 11 drugs, all of which showed an affinity for melanin. The highest melanin-binding ratio was seen in aminoglycosides. Among these, sisomicin sulfate (SISO) had the highest binding ratio (95.5%). The melanin-binding ratios seen in sulbenicillin sodium (SBPC), cephalosporins and fluoroquinolones were relatively low

during the early phase of the reaction, but increased with time. The highest ratio seen in cefazolin sodium was 60.1%. Melanin-bound aminoglycosides showed a reduction in their antibacterial activity with both Bacillus subtilis and Escherichia coli. The reduction rate in the antibacterial activity of melanin-bound SISO against B. subtilis was 50.0% and that against E. coli was 43.0%. No changes in antibacterial activity were seen in the other 6 drugs bound to melanin.

- **Montefiori DC, Modliszewski A, Shaff DI, Zhou J.**
Inhibition of human immunodeficiency virus type 1 replication and cytopathic effects by synthetic soluble catecholamine melanins in vitro. **Biochem Biophys Res Commun 168:200-205, 1990.**
Abstract : Synthetic sol. melanins were synthesized by spontaneous oxidn. of L-dopamine, norepinephrine or 5-hydroxytryptamine in weak alk. soln. These 3 melanins inhibited infection of human CD4+ lymphoblastoid cells (MT-2) by cell-free human immunodeficiency virus type 1 (HIV-1), without cell toxicity, at 0.15-10 .mu.g/mL. Also, syncytium formation and the resulting cytopathic effects when uninfected cells were mixed with HIV-1-infected cells were blocked by these melanins. Antisyncytial activity was greater when infected cells were preincubated with melanin than when uninfected cells were preincubated with melanin, suggesting that interaction of melanin with viral proteins is an important aspect of the antiviral mechanism.

- **Oyama Y.**
Skin-lightening preparations containing 10-pentadecenoic acid or 9-hexadecenoic acid. **Jpn Kokai Tokkyo Koho, 3 pp., 1990.**
Abstract : Skin prepns., which inhibit melamin formation and give no damage to the skin, contain 10-pentadecenoic acid (I) or 9-hexadecenoic acid as an active ingredient. A cream was prepd. from I 0.5, poly(oxyethylene) stearyl ether 2.0, poly(oxyethylene) cetyl ether 2.0, beeswax 6.0, cetanol 6.0, isoPr palmitate 10.0, liq. paraffin 30.0, polyethylene glycol monostearate 1.0, Me p-hydroxybenzoate 0.2, and H2O to 100%.

- **Sugita T, Ishiwata H, Yoshihira K, Kozaki M, Maekawa A.**
Melanin inhibits growth of microorganisms. **Shokuhin Eiseigaku Zasshi 31:187-188, 1990.**
Abstract : Melanin inhibited the growth of strains of Staphylococcus aureus, Saccharomyces cerevisiae, Bacillus cereus, Salmonella typhimurium, and Escherichia coli. The growth of these microorganisms in the presence of 10 mM melanin was 6.6-89% of that in the absence of melanin. The highest inhibition was obsd. with S. aureus. Streptococcus lactis was not inhibited.

- **Takeda Y, Suzuki A, Yamamoto H.**
Histopathologic study of epithelial components in the connective tissue wall of unilocular type of calcifying odontogenic cyst. **J Oral Pathol Med 19:108-113, 1990.**
Abstract : Histopathologic study of satellite cysts and odontogenic epithelial islands in connective tissue wall of unilocular type of calcifying odontogenic cyst (COC) was made. The material was 13 cases consisting of 3 simple unicystic COCs, 9 odontome producing COCs and 1 ameloblastomatous proliferating COC. Satellite cysts were found in 6 cases, and were histologically classified into following types: simple cystic, odontome producing and ameloblastomatous. Histologic types of satellite cysts did not coincide with those of main cystic lesions in some cases. Odontogenic epithelial islands with or without proliferating feature were found in 9 cases, and were found in all cases with satellite cysts. Melanin and melanocytes were seen in an ameloblastomatous satellite cysts of 1 of 3 pigmented COCs.

NEWS FROM THE ESPCR

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

Prof. Benvenuto GIANNOTTI
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Prof. Gunter SCHUTZ
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NB : In the last issue of the Bulletin, the name of the following member was not included. Please accept our apologies.

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Dept of Dermatology
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Meibergdreef 9
NL - 1105 AZ Amsterdam zuidoost

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ANNOUNCEMENTS

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ANNOUNCEMENTS & RELATED ACTIVITIES

Melanoma '91
The Brighton International Melanoma Conference
May 8th-11th 1991

PROGRAMME

WEDNESDAY 8th MAY

Registration
Trade Exhibition
Poster display

2.00 Opening Ceremony

Session 1 : The Cancer Research Campaign Symposium
"Public Education and Melanoma"
Chairman : Gordon McVie, CRC, London

Speakers : Dr Robin Graham Brown, Leicester
Dr Alan Marsden, St George's Hospital, London
Dr Andrew Warin, Exeter
Dr John White, Southampton
Prof John Hunter, Edinburgh
Prof Jocelyn Chamberlain, Thames Cancer Registry

G.P. Workshop 1 - "Clinical Diagnosis of Melanoma"

Biopsy Slide Seminar 1 - "The Diagnosis of Melanoma"

6.30 Civic Reception with the Mayor of Brighton
Viewing of Trade Exhibition and Poster Display

THURSDAY 9th MAY

Biopsy Slide Seminar 2 - "Prognostic Factors and Melanoma"

Session 2 : "Clinical Recognition of Melanoma"
Organized by Mr Per Hall, McIndoe Research Laboratory, Queen Victoria Hospital, East Grinstead

Speakers : Prof Rona MacKie, Glasgow
Dr Natale Cascinelli, Milan
Dr Gina Curley, Liverpool
Dr Anthony du Vivier, London

Session 3 : "Family Studies of Melanoma"
Organized by Dr Julia Newton
The Royal London Hospital

Speakers : Dr Julia Newton, London
Dr Wilma Bergen, The Netherlands
Dr David Elder, Philadelphia
Dr Bruce Ponder, Cambridge

LUNCH

Session 4 : "Surgery of Melanoma"
Organized by Professor Leslie Hughes, Cardiff

Speakers : Prof A. Bernard Ackerman, New York
Prof Leslie Hughes, Cardiff
Ms Judy Evans, Plymouth

Proferred Papers and Poster Viewing

G.P. Workshop 2 - "Pathology of Melanoma"

Biopsy Slide Seminar 3 - "Simulants of Malignant Melanoma"

FRIDAY 10th MAY

Biopsy Slide Seminar 4 - "Dysplastic Naevi"

Session 5 : "Dermatopathology of Dysplatic Naevi"
Organized by Dr Nigel Kirkham
Royal Sussex County Hospital, Brighton

Speakers : Prof A. Bernard Ackerman, New York
Dr David Elder, Philadelphia

Session 6 : "Limb Perfusion and Systemic Treatment"
Organized by Mr R. David Rosin
St Mary's Hospital, London

Speakers : Dr Natale Cascinelli, Milan
Dr Heimen Schraffordt Koops, Groningen
Mr R. David Rosin, London
Mr J. Meirion Thomas, London

LUNCH

Session 7 : European Society for Pigment Cell Research Symposium
Organized by Professor Patrick Riley, London

Speakers : Prof Giuseppe Prota, Naples
Dr Ghanem Ghanem, Brussels
Dr Bengt Larsson, Uppsala
Dr E. Link, London
Dr S. Pavel, The Netherlands
Prof Patrick Riley

Proferred Papers and Poster Viewing

G.P. Workshop 3 - "Differential Diagnosis"

Biopsy Slide Seminar 5 - "The Bloomsbury & Wessex Seminar"

8.00 Conference Dinner - Old Ship Hotel

SATURDAY 11th MAY

Biopsy Slide Seminar 6 - "The Melanoma Club Seminar"

Session 7 : "Oncology and Melanoma"
Organized by Professor Barry Hancock, Sheffield

Speakers : Dr Steven Rosenberg, Maryland
Dr R.C. Rees, Sheffield

G.P. Workshop 4 - "Management and Follow-up of Melanoma"

Melanoma Club Ten Year Celebrations

1.00 Closing Ceremony

Further information about this Conference can be obtained from Dr Nigel Kirkham, Consultant Pathologist, Department of Histopathology, Royal Sussex County Hospital, Brighton BN2 5BE, U.K.

RECALL

Dear Reader,

We are preparing a special issue of the Bulletin entirely dedicated to informations about the pigment cell research in Europe.

You will find on next page a standard form which will be published as such about your group filled by yourself. Also, feel free to photocopy this form as many times as there are different teams or/and programs. Should you know other teams working in pigment cell research, please send them a copy of the form or provide us with their name and address.

May we emphasize that not filling and sending back the form would mean that you will not be included in this special issue.

Thank you in advance for your cooperation.

G. PROTA
President

SURVEY OF CURRENT PIGMENT CELL RESEARCH IN EUROPE
1990 - 1991

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Send back to : Dr G. Ghanem, Assistant Editor
L.O.C.E. - Institut Bordet
Rue Héger-Bordet 1
B - 1000 Brussels

The 3rd Meeting of the European Society for Pigment Cell Research

will be held from September 8-11, 1991 in Amsterdam, The Netherlands.

Venue : Academisch Medisch Centrum
Medical Faculty
University of Amsterdam
Meibergdreef 9
NL - 1105 AZ Amsterdam

The program includes 3 guest lectures, symposia, plenary sessions, workshops and poster sessions.
Contact with the industries can be made at the commercial exhibition.

The topics to be addressed include:

Melanin : neuromelanins, biophysics, biochemistry

Melanocytes : culture methods, morphology, immunology, biology, biochemistry

Pigment disturbances : vitiligo, naevi, melasma, etc

Melanotropins : function, MSH-receptors

Melanoma : markers, growth factors, oncogenes, immunology, therapy

Sunscreens : natural, artificial

UV-light and pigmentation : skin types, tanning, colour measurement

Information : Dr. Wiete WESTERHOF
Chairman 3rd meeting ESPCR
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