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Discussion

Current surgical management of limb malignant melanoma

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The unpredictable natural history of malignant melanoma renders it unusual among human tumours. The tendency to arise from a benign lesion, its capacity to spread without regard for anatomical boundaries, its ability to lie dormant for many years and finally its tendency for spontaneous regression contribute to our lack of understanding of melanoma and our inability to deal with it more effectively.

The incidence of malignant melanoma is rising worldwide at a rate of 6% - 7% per year which is faster than any other cancer[1]. In Scotland (population of 5 million), 588 cases were registered in 1992, an increase from 507 in 1991. Approximately 50% of all melanomas occur on the extremities and a large percentage of these occur in women[2]. Multivariate analysis of prognostic variables has shown that the most important prognostic factors are pathological stage, ulceration, thickness and site.

Since Handley delivered his Hunterian lecture[4] in 1907, the treatment of malignant melanoma has been almost exclusively surgical, and based on wide excision of the primary tumour with or without regional lymphadenectomy.

For many years wide excision margins were recommended to remove occult foci of melanoma cells which could lead to metastasis or local recurrence. Current practise is to excise the primary malignant melanoma with surgical margins that are based on tumour thickness and ulceration, as these factors correlate with the risk of local recurrence[5,6]. It is known that the incidence of local recurrence or metastasis from melanoma less than 0.76mm thick is not affected by the width of the excision margin[7].

The World Health Organisation (WHO)[8] randomised study of 612 patients in whom excision was randomised to 1 or 3 cm, demonstrated that a 1 cm margin was adequate for primary melanoma less than 1mm thick. The optimal excision margin for melanoma thicker than 1mm is still controversial[9,10]. Indeed, a further randomised study is presently being conducted on excision margins in the United Kingdom (Meirion Thomas, Royal Marsden Hospital, London).

Our current recommendations for surgical margins are as follows:

In situ	local excision
<1mm thick	1 cm
1-3mm thick	2 cm
> 3mm	3 cm (if cosmetically possible)

The site of the primary lesion clearly has an important bearing on excision margins.

The surgical world still has considerable difficulty in knowing how to deal with lymph nodes which may not contain metastatic malignancy. Most would agree that involved lymph nodes should be removed, but what if nodes are not clinically involved and yet contain micrometastases? Such a situation is probably more common than has been hitherto realised and has important implications for the staging of disease and interpretation of therapeutic trials.

Those surgeons who advise immediate elective lymph node dissection in all patients with high risk malignant melanoma (Breslow 1.5 - 4.0mm) must accept that many patients will undergo an unnecessary operation which is associated with significant morbidity[11.12]. Others adopt a 'wait and see' policy and remove lymph nodes if and when they become clinically palpable[13,14]. Neither policy is ideal, but around the world enthuasium for elective lymph node dissection is waning[15].

If a technique could be developed that would allow positive identification of the subgroup of patients with clinically occult nodal disease, it may well be that such patients would be most likely to benefit from lymphadenectomy. Lymphatic mapping first described by Morton[16] and Cochran[17] may be such a technique.

Intraoperative lymphatic mapping has been developed on the assumption that metastases embolise via lymphatic channels to the regional lymph nodes. The technique is based on identifying the first or sentinel lymph node (the draining lymph node nearest the primary melanoma) and examining it for micrometastases. The method depends on using a dye to stain this sentinel node.

Patent blue dye is injected around the site of the primary melanoma, the regional lymphatic basin is explored and the blue lymphatic channels are followed to the first stained blue node. We have performed this technique in 40 patients with clinically stage I disease. Sentinel nodes were identified in all patients and nine nodes were found to contain micrometastases.

Our pilot study revealed that it was not possible to predict the status of the sentinel node from the Breslow thickness of the primary tumour. This is perhaps surprising as the proponents of elective lymphadenectomy claim that the risk of developing regional node disease increases with the thickness of the primary melanoma and propose that elective lymph node dissection is most beneficial in those patients with intermediate thickness tumour (1.5-4.0mm)[18].

However, tumour ulceration did appear to be significant in the prediction of sentinel node status. Seven of the nine patients with positive sentinel nodes had ulceration in the primary tumour while only one of the thirty-one patients with negative sentinel nodes had ulceration in the primary tumour.

Our initial experience of this technique suggests that it is sensitive and easy to master. It may allow us to reserve lymphadenectomy for patients in whom metastases are positively identified. A multicentre study is currently in progress on the role of lymphatic mapping in malignant melanoma. The WHO are also proposing a multicentre study of the technique. The exact role of intraoperative lymphatic mapping must await the outcome of these studies but our initial experience does show that it is practical and allows detection of clinically unsuspected disease.

Since 1984, we have been using isolated limb perfusion in the management of limb malignant melanoma. In a study of 120 patients with lesions greater than 1.5mm (mean thickness 3.1mm) we have been unable to show survival benefits for adjuvant isolated limb perfusion using melphalan and mild hyperthermia (<40°C). Though there is a trend in favour of the isolated limb perfusion group both in terms of survival and time to first recurrence, this does not reach statistical significance. This finding has been supported by a prospective study of isolated limb perfusion being conducted by the members of the EORTC melanoma subgroup. At the present time it is not possible to recommend routine adjuvant isolated limb perfusion using melphalan for patients with limb lesions more than 1.5mm.

We continue to use isolated limb perfusion for patients with recurrent melanoma of the extremities. In more than 180 patients, we are able to achieve a complete response of 80% and a partial response of 19%. These results are comparable with those being achieved in other centres[19,20].

When isolated limb perfusion is not possible, or has failed to achieve disease control, the option for disease control includes isolated limb perfusion with melphalan and tumour necrosis factor, or laser ablation. The addition of tumour necrosis factor to the perfusion circuit has produced encouraging results, though the morbidity of the procedure rises[21]. Our own experience of laser ablation is limited to 19 patients but we are convinced that it is a

useful means of achieving local control and has the great benefit of being a relatively minor procedure which can be readily repeated[22].

Despite the vast increase in our knowledge of melanoma in all its facets, it seems extraordinary that the prognosis for an individual patient given appropriate surgery, has not changed since 1907. If improvement of disease control and prognosis is to be achieved, then targeting and destroying subclinical melanoma metastases must be the way forward. We have shown that in animal studies the use of targeted radiotherapy is effective in killing melanoma cells. Other investigators have reported the value of melanoma cell vaccincs[24].

At present, sensible local excision and more accurate staging is possible. Local disease control is usually achievable. Adequate treatment for disseminated disease remains elusive.

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combination with interferon and melphalan in isolation perfusion. World J Surg 1992; 16:234-240.
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CURRENT LITERATURE

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

- Herve M, Hirschinger J, Granger P, Gilard P, Deflandre A, Goetz N.
A 13C solid-state NMR study of the structure and auto-oxidation process of natural and synthetic melanins. Biochim Biophys Acta 1204(1):19-27, 1994.
Abstract: This paper presents a 13C CP/MAS NMR study of the melanin pigments obtained through natural and synthetic origins: sepia-melanin from squid ink and three synthetic 5,6-dihydroxyindole- melanins prepared using different non-enzymatic oxidation pathways. The synthetic pigments can be distinguished from natural melanin by the absence of aliphatic carbons, thereby confirming the unreacted 3,4-dihydroxyphenylalanine and the proteinaceous origins of the aliphatic resonances in natural eumelanin. The spectra of selected non-protonated carbon resonances and those with only protonated carbon signals led to a quantitative analysis. An auto-oxidative experiment using a synthetic melanin , over a period of 130 h, has shown an unusually slow disappearance of hydrogen peroxide formed in situ. The 13C-NMR spectrum of the insoluble oxidized synthetic melanin compared to that before auto-oxidation clearly demonstrates that the oxidation process is associated with chemical changes within the pigment; i.e., carbonyl functional group formation and an increase of the non-protonated carbons fraction.

- Rosei MA, Mosca L, Coccia R, Blarzino C, Musci G, De Marco C.
Some biochemical properties of melanins from opioid peptides. Biochim Biophys Acta 1199(2):123-9, 1994.
Abstract: Opioid peptides are converted by mushroom tyrosinase into melanin -like compounds retaining the peptide moiety (opio-melanins). Opio- melanins, owing to the presence of the linked aminoacids and in contrast with DOPA- melanin , are soluble compounds. The enkephalin-generated melanins are cleaved by carboxypeptidase A and pronase whereas aminopeptidase M cannot remove aminoacids from the pigment. Enkephalins, as well as other opioid peptides, (alpha-endorphin, kyotorphin, esorphins) if oxidized in presence of DOPA and tyrosinase are readily incorporated into DOPA- melanin . The resulting mixed- melanins (opio- melanin + DOPA- melanin) can be solubilized in hydrophilic solvents. Melanin from leu-enkephalin exhibits paramagnetism as evidenced by an EPR spectrum identical to that of DOPA- melanin, but unlike the latter pigment, it does not appear to oxidize NADH, probably for the presence of the peptide moiety that exerts a hampering effect on the oxidizing capacity.

- Salinas C, Garcia-Borron J-C, Solano F, Lozano JA.
Dopachrome tautomerase decreases the binding of indolic melanogenesis intermediates to proteins. Biochim Biophys Acta 1204(1):53-60, 1994.
Abstract: Dopachrome tautomerase (DCT) is a recently characterized enzyme contributing to the control of melanogenesis in mammals. The enzyme catalyzes the rearrangement of L-Dopachrome (L-DC) to 5,6-dihydroxyindole 2-carboxylic acid (DHICA), while the spontaneous rearrangement of L-DC leads to 5,6-dihydroxyindole (DHI). Due to the lower reactivity of DHICA in comparison to DHI, DCT could provide a protective mechanism against the cytotoxicity of decarboxylated indolic melanogenic intermediates by limiting the formation of these highly reactive decarboxylated species within melanocytes. We have followed the binding of radioactive melanogenic precursors to a model protein, bovine serum albumin (BSA). Using L-DC as initial melanin precursor, this binding was decreased by DCT in a concentration-dependent manner. In the presence of tyrosinase, the binding of L-Dopa-derived intermediates to BSA was also decreased by DCT and the percentage of decrease was even higher than using L-DC as initial melanin precursor. SDS-PAGE followed by fluorographic detection of radioactive bands showed the formation of covalent adducts between BSA and melanin precursors, as well as of aggregated forms of this protein. This aggregation was also diminished by DCT. These data indicate that DCT could play a protective role against the cytotoxic action of decarboxylated indoles within mammalian melanocytes.

-Seraglia R, Traldi P, Elli G, Bertazzo A, Costa C, Allegri G.
Laser desorption ionization mass spectrometry in the study of natural and synthetic melanins. I--Tyrosine melanins. Biol Mass Spectrom 22(12):687-97, 1993.

Abstract: Nine synthetic, biosynthetic and natural tyrosine melanins , together with dopa and catechol melanins , have been studied by means of laser desorption ionization mass spectrometry. The results obtained have shown that all the melanins under study consist of homogeneous sets of oligomers and have led to definitive information on their molecular weight distribution (from 500 to 30,000 Da). A deeper analysis of the spectra, in terms of mass differences among the various oligomers, allowed the identification of some monomeric units, identical or analogous to those already proposed in the literature.

- Shinkichi Honda, Yoichiro Takekoshi, Yumiko Nakano, Yoichiro Arai.
Bio-Melanin (as a new cosmetic raw material). J. SCCJ 27:432-440, 1993.
Abstract: Recently a great deal of attention has been paid to the prevention of photo-aging, many kinds of UVA, UVB-absorbents and micro fine powders have been developed as new cosmetic raw materials. The authors have recently developed a new type of melanin, Bio-Melanin which is produced by microorganism Streptomyces sp. Bio-Melanin is obtained at a high purity without protein contamination after simple purifn. techniques. Bio-Melanin has low toxicity, and low photocontact sensitivity. Bio-Melanin showed protective effects on cultured mouse fibroblast cells (L929) against UVB irradn. Bio-Melanin is alk. sol. natural UV-absorber and is suited for use in new cosmetic powders and Bio-Melanin-coated powders (TiO2, sericite, mica etc.). This type of cosmetic powder (Bio-Melanin-coated powder) has dual function and reflection and can be used in the prodn. of foundation, lip-cream, eye liner and skin care products. The sunscreen cream contg. BIO-MELANIN-coated powder had a high SPF value.

- Sotomatsu A, Tanaka M, Hirai S.
Synthetic melanin and ferric ions promote superoxide anion-mediated lipid peroxidation. FEBS Lett 342(2):105-8, 1994.
Abstract: In this study, we demonstrate that synthetic dopa- melanin produced superoxide anions and promoted the peroxidative cleavage of phospholipids in the presence of Fe(3+)-ADP complexes. SOD significantly suppressed this lipid peroxidation, while catalase or sodium benzoate did not. During the reaction, MCLA-dependent chemiluminescence was detected, and this was suppressed to the control level by the addition of SOD. Melanin has been postulated to be toxic to tissues because of its interaction with redox-active paramagnetic metal ions, and these findings suggest that superoxide anion-mediated lipid peroxidation is induced by melanin in the presence of iron.

-Urabe K, Aroca P, Tsukamoto K, Mascagna D, Palumbo A, Prota G, Hearing VJ.
The inherent cytotoxicity of melanin precursors: a revision. Biochim Biophys Acta 1221(3):272-8, 1994.
Abstract: The potential cytotoxicity of the melanogenic intermediates DOPA, (L-3,4-dihydroxyphenylalanine) and DHI (5,6-dihydroxyindole) has long been recognized and exploited as a targeting concept in experimental melanoma therapy. In recent years, however, a novel branchpoint in the melanin biosynthetic pathway has been shown to divert the metabolism of DOPAchrome to a carboxylated derivative termed DHICA (DHI-2-carboxylic acid) rather than to DHI. In order to evaluate the biological implications of this regulatory control, we have reexamined the inherent cytotoxicity of DHICA versus DHI on different cell lines. We found that under the usual conditions of the biological assay, the apparent cytotoxicity of the two indoles reflect their instability in the culture medium, the less stable DHI being generally more toxic than DHICA to melanoma cells and nonmelanocytic cells. Moreover, the observed cytotoxic effects increased with the time of incubation and were markedly reduced by the addition of catalase to the medium, suggesting that they were probably due to the generation of reactive oxygen species (particularly H2O2) during the autoxidation of the melanin precursors outside the cells. To circumvent this problem, we then tested the diacetylated derivatives of DHI and DHICA (DAI and DAICA) which are sufficiently stable until taken up into the cells whereupon they may be converted by endogenous esterases back to the parent indoles. Although DAI proved to be cytotoxic for nonmelanocytic cells, it had no detectable activity on melanoma cells, whereas DAICA showed no effect on any of the cells examined. These results, when combined with other studies, point to a reconsideration of the inherent cytotoxicity of the 5,6-dihydroxyindoles, as well as DOPA, to melanin producing cells.

- Yoichi A.
Oxidation of catecholamines in insects. Nippon Noyaku Gakkaishi 19(2),S61-S65, 1994.
Abstract: A review with 13 refs. on formation of melanin from dopa, enzymes involved in the process, and hardening of cuticle by oxidn. of dopamine derivs.

- Zajac GW, Gallas JM, Cheng J, Eisner M, Moss SC, Alvarado-Swaisgood AE.
The fundamental unit of synthetic melanin: a verification by tunneling microscopy of X-ray scattering results. Biochim Biophys Acta 1199(3), 1994.
Abstract: A characteristic dimension of a melanin protomolecule synthesized from tyrosine has been investigated by scanning tunneling microscopy (STM). Identification of a melanin protomolecule of approximately approximately 20 A lateral extent and approximately 10 A height has been established. This size is in good agreement with models constructed to fit wide angle X-ray diffraction experiments on melanin . These protomolecules are believed to consist of Van der Waals interacting stacks of a basic random polymer of 5.6 indolequinone units. There is extensive pi-delocalization within the individual polymeric sheets. Structure minimization and molecular orbital techniques were employed to verify the X-ray and STM results.

- Zhang F, Dryhurst G.

Effects of L-cysteine on the oxidation chemistry of dopamine: new reaction pathways of potential relevance to idiopathic Parkinson's disease. J Med Chem 37(8):1084-98, 1994.
Abstract: Oxidation of the catecholaminergic neurotransmitter dopamine (1) at physiological pH normally results in formation of black, insoluble melanin polymer. In this study, it is demonstrated that L-cysteine (CySH) can divert the melanin pathway by scavenging the proximate o-quinone oxidation product of 1 to give 5-S-cysteinyldopamine (8). This cysteinyl conjugate is further oxidized in the presence of free CySH to give 7-(2-aminoethyl)-3,4- dihydro-5-hydroxy-2H- 1,4-benzothiazine-3-carboxylic acid (11) and its 6-S-cysteinyl (12), 8-S-cysteinyl (14), and 6,8-di-S-cysteinyl (16) conjugates in addition to many other unidentified compounds. 5-S-Cysteinyldopamine (8) and dihydrobenzothiazines 11, 12, 14, and 16 are all more easily oxidized than 1. With increasing molar excesses of CySH, the formation of melanin is decreased and, ultimately, completely blocked. Preliminary experiments have revealed that when injected into the brains of laboratory mice, dihydrobenzothiazine 11 and its cysteinyl conjugates 12 and 14 are lethal and evoke profound behavioral responses including hyperactivity and tremor. On the basis of these results and other recent observations, a new hypothesis has been advanced which might help explain the selective degeneration of nigrostriatal dopaminergic neurons which occurs in idiopathic Parkinson's Disease (PD). This hypothesis proposes that in response to some form of chronic brain insult, the activity of gamma-glutamyltranspeptidase is upregulated leading to an increased rate of translocation of glutathione (GSH) into the cytoplasm of dopaminergic cell bodies in the substantia nigra (SN) para compacta. The results of this in vitro study predict that such an elevated translocation of GSH into heavily pigmented dopaminergic neurons would cause a diversion of the neuromelanin pathway with consequent depigmentation of these cells and formation of 8, all of which occur in the Parkinsonian SN. The further very facile oxidation of 8 which must occur under intraneuronal conditions where 1 is autoxidized, i.e., in neuromelanin-pigmented cells, would lead to dihydrobenzothiazine 11 and its cysteinyl conjugates which could be the endotoxins responsible for the selective degeneration of dopaminergic SN neurons in PD. The ease of autoxidation of 8 is suggested to account for the low levels of this conjugate found in the degenerating and Parkinsonian SN.

2. Biology of pigment cells and pigmentary disorders

-Abdel-Malek Z, Swope V, Collins C, Boissy R, Zhao H, Nordlund J.
Contribution of melanogenic proteins to the heterogeneous pigmentation of human melanocytes. J Cell Sci 106(Pt 4):1323-31, 1993.
Abstract: Human melanocytes from individuals with different skin types, as well as from the skin of the same individual, are heterogeneous in their melanin content. This heterogeneity may be attributed to differences in the activity and expression of the three melanogenic proteins: tyrosinase and tyrosinase-related proteins 1 and 2 (gp75 and DOPAchrome tautomerase, respectively), which in turn are affected by certain regulatory factors. Established melanocyte strains that exhibited intrinsic melanogenic heterogeneity could be separated into subpopulations according to density and melanin content by Percoll density gradient centrifugation. The least melanotic subpopulation consisted of melanocytes that contained an active tyrosinase enzyme and a low amount of melanin. Tyrosinase activity and the quantities of tyrosinase enzyme, tyrosinase-related protein-1 and DOPAchrome tautomerase gradually increased with increased melanin content and Percoll density of the isolated melanocyte subpopulations. We have found a direct correlation between melanin content, tyrosinase activity and the expression of the three melanogenic proteins in melanocyte strains established from different skin types. Addition of the two epidermal cytokines, tumor necrosis factor-alpha or interleukin-1 alpha, to cultures of human melanocytes from different skin types caused decreased proliferation, tyrosinase activity and expression of tyrosinase, tyrosinase-related protein-1 and DOPAchrome tautomerase. Similar results were obtained when Percoll-derived melanocyte subpopulations were treated with tumor necrosis factor-alpha and interleukin-1 alpha. These results indicate that the variation in melanin content in human melanocytes is due to differences in the activity and expression of the melanogenic proteins, which are influenced by autocrine and paracrine factors.

- Aquaron R.
Human oculocutaneous albinism. From clinical observation to molecular biology. Bull Soc Pathol Exot 86(5):313-26, 1993.
Abstract: Human oculocutaneous albinism (OCA) is a heritable metabolic defect transmitted as an autosomal recessive trait and characterized by a hypopigmentation of skin, hair and eyes. This defect is mainly due to an altered or absence of tyrosinase activity, the key enzyme of eu-and pheo- melanin synthesis. It is a seldom condition in white peoples but more frequent in Africans and in Afro-Americans. Albinos, especially in tropical settings, have high prevalence of solar keratosis and squamous cell carcinoma. Ocular defects characteristics of OCA are photobia, nystagmus and decreased visual acuity. Two forms of OCA have been distinguished in 1970 on the basis of their genetic, clinical, biochemical and ultrastructural characteristics: type I i. e. tyrosinase negative and type II i. e. tyrosinase positive. Actually 10 forms are described. Human tyrosinase gene has been mapped to chromosome 11 (q14-21) and cloned. It is formed by 5 exons. Several different human tyrosinase gene mutations have been identified in patients with type I-A OCA. Non sense, misense and frameshift mutations result in altered or absence of tyrosinase activity.

-Conlee JW, Gerity LC, Westenberg IS, Creel DJ.
Pigment-dependent differences in the stria vascularis of albino and pigmented guinea pigs and rats. Hear Res 72(1-2):108-24, 1994.
Abstract: Functional models of the stria vascularis (SV) have ascribed roles for the marginal and basal cells, but not for the intermediate cells, which remain poorly understood. Intermediate cells have been identified as melanocytes, which produce melanin in most pigmented

animals including humans. The relationship of melanin to intermediate cell function may be addressed through comparisons with the albino inner ear. Albinos have a normal distribution of melanocytes that are unable to synthesize melanin pigment. In the present study, the SV was compared between albino and pigmented littermates in both the guinea pig and the rat. Photomicrographic montages of the SV were analyzed from each of 7 cochlear regions in the guinea pig and 5 regions in the rat. Stereological procedures were used to determine the volume density (Vv) for each of the three main cell types in the stria, the surface density (Sv) of the marginal cells, and to derive estimates of absolute cell volume and surface area. In the guinea pig, comparisons between pigment groups showed that marginal cell Vv was larger across cochlear turns in the albinos, while intermediate cell Vv was smaller. Intermediate cell cytoplasmic and total cell volumes were smaller in the albino guinea pigs; however, marginal cell Sv and absolute area were larger. In the rat, intermediate cell Vv was alos smaller across cochlear turns in the albinos. Similarly, intermediate cell cytoplasmic and total cell volumes were smaller in the albinos, while marginal cell total surface area per radial cross-section of the SV was larger. These results demonstrate that amelanotic melanocytes occupy significantly less volume than do pigmented melanocytes, and suggest that melanin may influence the structure and function of the SV.

- Hegedus ZL, Nayak U.
Melanin quantitation from human erythrocytes; interference by haeme derivatives. Arch Int Physiol Biochim Biophys 101(5):289-95, 1993.
Abstract: Precipitates were obtained by 6 N HCl hydrolysis of human erythrocytes and subsequent extractions with ethanol-ether 1:1 and with tetrahydrofuran. The mean quantity of these precipitates (n = 16) was 16.60 +/- 1.60 (standard deviation) mg/ml, (15.09 +/- 1.45 mg/g) and from saline washed erythrocyte samples (n = 8) 16.65 +/- 0.73 mg/ml, (15.14 +/- 0.67 mg/g). A large part of these precipitates (about 74%) is associated with haemoglobin (in average 12.34 mg/ml). Melanins account for the difference (16.60-12.34) = 4.26 mg/ml, approximately 8.7% of haemoglobin-free erythrocyte solids. Precipitates from red cells, and from haemoglobin produced similar UV-VIS and IR spectra. The precipitates from haemoglobin are mainly derivatives of haeme (about 97%); the remaining approximately 3% are melanins from globin. The total melanins are about 1.3% of haeme-free solids of erythrocytes. Precipitation from the erythrocytes with 6 N HCl was also achieved in practically complete argon atmosphere, and similar quantities were obtained as those in air with the same UV-VIS and IR spectra. Since granules or solid particles are not found in the cytoplasm of normal human erythrocytes, we conclude that soluble melanins are present. Small amounts of melanins can be present in the membranes as well, since the precursors of melanins : norepinephrine, epinephrine are present in these membranes.

- Koch PB.
Wings of the butterfly Precis coenia synthesize dopamine melanin by selective enzyme activity of DOPA decarboxylase. Naturwissenschaften 81:36-38, 1994.
Abstract: The authors show that tyrosine can serve as the only melanin precursor in isolated wings in vitro. Enzyme inhibitors added to the culture medium show that wings metabolize tyrosine via DOPA to dopamine as a precursor of melanin formation. The distribution of DOPA decarboxylase, which converts DOPA to dopamine, demonstrates that pattern-specific melanin formation is regulated by pattern-specific enzyme activity in the butterfly.

- McLeod SD, Ranson M, Mason RS.
Effects of estrogens on human melanocytes in vitro. J Steroid Biochem Mol Biol 49(1):9-14, 1994.
Abstract: Subjects with elevated serum estrogen concentrations, such as those who are pregnant or ingesting estrogen-containing contraceptive medication, may develop increased skin pigmentation. As little information is available on the mechanism(s) underlying this relationship, the in vitro effects of estrogens on melanocytes cultured from normal human skin were examined. Physiological concentrations of 17 beta-estradiol (10(-11) to 10(-9) M) significantly increased the activity of tyrosinase in melanocytes from 15 of 23 subjects. The observed increases ranged from 1.2- to 2.4-fold. Melanin synthesis, which correlated with tyrosinase activity ($r = 0.98$, $P < 0.001$) was increased to a similar extent. Melanin extrusion was also increased by 17 beta-estradiol (10(-9) M). The estrogens, estriol (10(-9) M) and estrone (10(-9) M) stimulated tyrosinase activity and melanin extrusion to a lesser extent than 17 beta-estradiol. The analogue 17 alpha-estradiol (10(-9) M) was shown to have effects on melanocyte tyrosinase activity and melanin extrusion that were equivalent to those of 17 beta-estradiol. The pure estrogen antagonist ICI 164384 (10(-6) M) also stimulated tyrosinase activity. Cycloheximide (50 micrograms/ml) inhibited 17 beta-estradiol-induced tyrosinase stimulation ($P < 0.001$). These results indicate that several aspects of melanocyte function respond directly to estrogenic stimulation. The equivalent effects of the 17 alpha-analogue and a "pure" anti-estrogen suggest that the 17 beta-estradiol response may be mediated through a non-classical mechanism which is similar to that described in other tissues of neural crest origin.

-Miranda M, Amicarelli F, Poma A, Ragnelli AM, Scirri C, Aimola PP, Masciocco L, Bonfigli A, Zarivi O.
Cyto-genotoxic species leakage within human melanoma melanosomes, molecular-morphological correlations. Biochem. Mol. Biol. Int. 32:913-922, 1994.
Abstract: This work studies the phenotype changes, relating to pigment expression, of a human melanoma cell line. The phenotypic instabilities and proliferation rates are correlated with the prodn. and release in the cell culture medium of active oxygen species and melanin synthesis intermediates. The proliferation rates vs. L-tyrosine concn. in the culture media are investigated: a decrease is found

when high L-tyrosine is added to the medium. This would be consistent with the release of cytotoxic and/or genotoxic species by melanoma cells. The morphol. of melanoma melanosomes is coherent with the leakage of cytotoxic and genotoxic species produced during melanin synthesis.

-Sakai Itaru, Tanaka Tomohide, Morita Yutaka, Satoh Kana, Hibi Takashi, Ohsawa Sigemitsu, Nakajo Shigeo, Nakaya Kazuyasu.
Inhibitory effect of geranylgeranylacetone on melanogenesis. Biochemical study using cultured B16 melanoma cells. Nippon Hifuka Gakkai Zasshi 103:1851-1856, 1993.
Abstract: Inhibitory effects of (geranylgeranyl)acetone (GGA) and related compds. (geraniol, farnesol, geranylacetone, farnesylacetone, and gefaronate) on mushroom tyrosinase, and on cultured B16 melanoma cells were examd. These compds. had no inhibitory effect on mushroom tyrosinase activity. GGA at a nontoxic concn. (9.1 .times. 10-6 M) restrained the melanogenesis of cultured B16 melanoma cells by 20% to control cells. The ratio of eumelanin to pheomelanin in B16 melanoma cells treated with GGA was quantified to be 0.05; that of control cells was 0.22. No apparent effect on tyrosinase activity was detected in the presence of any of the other compds. Because the amt. of tyrosinase in B16 melanoma cells treated with GGA decreased, it is suggested that GGA inhibits tyrosinase synthesis in B16 melanoma cells.

- Summers CG, King RA.
Ophthalmic features of minimal pigment oculocutaneous albinism. Ophthalmology 101(5):906-14, 1994.
Abstract: PURPOSE: The purpose of this study is to describe the heterogeneous phenotype of individuals with an unusual type of albinism--minimal pigment oculocutaneous albinism. METHODS: Nine patients with minimal pigment oculocutaneous albinism were identified and followed for up to 11 years. The criteria were the presence of oculocutaneous albinism in association with low hairbulb tyrosinase activity in the patient and disparate activity in the parents with one parent having normal activity and the other having low tyrosinase activity. Changes in skin, hair, and ocular pigment were followed as the patients matured. As a measure of ocular pigment, iris transillumination and macular transparency were graded according to a previously published scheme. RESULTS: Patients were born with white scalp hair and skin, and nystagmus developed. Visual acuity was reduced to 20/50 to 20/200 for the group, but in one patient vision improved with maturity. Irides were blue. In seven patients, iris pigment developed, which was detected by transillumination with slit-lamp biomicroscopy, including the one patient with improved visual acuity. All patients had foveal hypoplasia, and melanin pigment in the fundi could not be detected by clinical examination. Visual acuity in the group did not correlate directly with the presence or development of iris transillumination or macular transparency. The pedigrees were consistent with an autosomal recessive inheritance pattern. CONCLUSION: This unique type of oculocutaneous albinism has heterogeneous clinical features. Minimal pigment oculocutaneous albinism appears to represent a new type of tyrosinase-related oculocutaneous albinism (OCA1MP).

- Takashi Horikoshi, Hiroaki Eguchi, Hideo Onodera.
The effects of tranexamic acid on growth and melanogenesis by cultured human melanocytes. Hifuka Gakkai Zasshi 104:641-646, 1994.
Abstract: The effects of transexamic acid (TA) on growth and melanogenesis by human melanocytes (MC) were studied. Cells were cultured in the presence of various concns. of TA for 14 days. The amt. of 3H-TdR (thymidine) incorporated into the cells decreased by 20-50% in the presence of 1-50 .mu.g TA/mL. At >5 .mu.g TA/mL, the amt. of 3H-TdR incorporated was greater than that with 1 .mu.g/mL. No inhibitory effect on the uptake of 3H-TdR was noted at TA concns. of 0.1-1 .mu.g/mL. Although TA may inhibit the growth of MC, its EC appears to be limited to a narrow range. There were no apparent changes in either tyrosinase activity or melanin content of cultured MC in the presence of 0.5-50 .mu.g TA/mL. The uptake of 14C-thiouracil was increased by TA in a concn.-dependent manner, suggesting that TA promotes melanin synthesis in MC.

- Yoshino K, Takahashi H, Torii S, Kakihana H.
Complex formation of p-boronophenylalanine with L-DOPA (a precursor of melanin metabolism) and oxidation reaction of the analogs. Kyoto Daigaku Genshiro Jikkensho Tech. Rep. KURRI-TR-382:33-8, 1993.
Abstract: P-boronophenylalanine-L-DOPA complex formation was confirmed in artificial body fluid at pH 7.4, as studied by 11B-NMR. The degrdn. products dopaquinone, boric acid, and phenylalanine of the complex were also identified after oxidn. The results are discussed with regard to the uptake mechanism of 10B in melanoma after treatment with p-boronophenylalanine for neutron capture therapy.

(Comments by Dr M. Picardo)

-Hideya Ando, Akira Hashimoto, Yukimitsu Masamoto, Masamitsu Ichihashi, Yutaka Mishima.
Inhibitory effect of linoleic acid on melanogenesis. J. SCCJ 27:415-423, 1993.

-Masato Tagawa, Tomoji Murata, Toshio Onuma, Koichiro Kameyama, Chie Sakai, Shigeo Kondo, Kozo Yonemoto, Quigley J, Dorsky A et al.
Inhibitory effects of magnesium ascorbate phosphate on melanogenesis J. SCCJ, 27:409-414, 1993.

Commentary: The possibility of interfering with the melanogenesis process is one of the main tools in the studies on pigmentation. In these studies the authors present in vitro and in vivo studies on the inhibition of melanogenesis by linoleic acid and magnesium ascorbate. Whereas the mechanism of linoleic acid inhibition is not completely clear, magnesium ascorbate probably acts trough the inhibition of tyrosinase activity. The effect of ascorbate on melanogenesis in vitro is well known, but the in vivo activity is limited because the rapid oxidation rate of ascorbic acid. The use of different salts seem to be a good alternative and an useful approach for an in vivo application.
Linoleic acid, which is an unsaturated acid commonly present in the epidermal lipids, could represent a natural modulating factor of melanogenesis. Free fatty acids posses different pharmacological activities in vitro systems and, through modifications of cell membranes, are able to interfere with different enzymatic activities. Moreover, it undergoes peroxidation following UV exposure and products of lipid peroxidation are known to be able to interfere with melanocyte and keratinocyte activities.

-Schallreuter KU, Wood JM, Ziegler I, Lemke KR, Pittelkow MR, Lindsey NJ, Gutlich M.
Defective tetrahydrobiopterin and catecholamine biosynthesis in the depigmentation disorder vitiligo. Biochim Biophys Acta 1226(2):181-92, 1994.

-Schallreuter KU, Wood JM, Pittelkow MR, Gutlich M, Lemke KR, Rodl W, Swanson NN, Hitzemann K, Ziegler I.
Regulation of melanin biosynthesis in the human epidermis by tetrahydrobiopterin. Science 263(5152):1444-6, 1994.
Commentary: From a couple of years the group of K. Schallreuter and J Wood is working on a new hypothesis of the occurence of vitiligo. The authors have shown that human keratinocytes are able to synthesise catecholamines and have suggested that an alteration of this metabolic pathway is involved in the disease. Now they report a defective metabolism of biopterin as the biochemical marker of vitiligo. In this patients is detectable an increase activity of GTP-cyclohydrolase I and a decreased activity of 4a-hydroxytetrahydrobiopterin dehydratase. These alterations lead to the abnormal formation of 7-dehydrobiopterin, an inhibitor of phenyl alanine hydroxylase, and to an increase of norepinephrine synthesis with an excess of the catecholamine level in both plasma and urine of these patients. The findings are interesting. However, it does not seem completely clarified the mechanism of melanocyte degeneration and the correlation between the clinical course (age of onset, active and stationary phases) of the disease and the constitutive enzymatic alterations.

-Slominski A, Paus R, Plonka P, Chakraborty A, Maurer M, Pruski D, Lukiewicz S.
Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle. J Invest Dermatol 102(6):862-9, 1994.
Commentary: Hair pigmentation is a fascinating model for the studies on the interactions between keratinocytes, melanocytes and dermal structures. In this paper the authors present the continuation of their studies on the hair cycle in mouse model. It is compared the EPR signals of melanin during the hair cycle with the expression and activity of melanogeneis related enzymes and the histologic appearance of the follicles. The results show the rapidly increase of melanogenesis related enzyme activities during early anagen and the decline in before telogen in both tyrosinase and dopachromo tautomerase activity and disappearance in immunoblot detectable proteins. The main findings are that probably during anagen a similar signals stimulate hair growth and melanogenesis and that factors inducing catagen are also able to interfere with melanocyte activity. Moreover, in this model, tyrosinase activity seem to be better correlated with the hair cycle than the other melanogenesis related enzymes.

(Comments by Dr N. Smit)

- Lontz W, Olsson MJ, Moellmann G, Lerner AB.
Pigment cell transplantation for treatment of vitiligo: a progress report. J Am Acad Dermatol. 30(4):591-7, 1994.
Commentary: In the paper by Lontz et al it is reported that transplantation of autologous melanocytes can result in repigmentation of skin of patients with vitiligo even when conventional therapeutic approaches have failed. After reports by Brysk (1989) and Ollson and Juhlin and Zachariae et al (1993) this study is another example that transplantation techniques of cultured mixed epidermal cells or pure melanocytes may offer an advantageous addition to the treatments already available for patients with vitiligo.

3. MSH, MCH, other hormones, differentiation.

-Cardot J, Fellmann D, Bugnon C.
Melanin-concentrating hormone-producing neurons in reptiles. Gen. Comp. Endocrinol. 94:23-32, 1994.
Abstract: Melanin-concg. hormone (MCH)-like producing neurons were mapped in the brains of several reptiles using antisera (AS) prepd. against salmon MCH (sMCH) and peptides derived from the rat MCH precursor (rMCH, NGE, NEI) or cross-reacting with these peptides (anti-GRF37 and anti-.alpha.-MSH). MCH neurons were detected in the periventricular and lateral hypothalamic nuclei. The coexpression of MCH-, GRF37-, and NEI-like immunoreactivities suggests that the reptile precursor presents large sequence homologies with the rat/human precursor. MCH neurons project to many brain areas, but fibers are very scarce in the median eminence, and the neurohypophysis is devoid of immunoreactive processes. Thus the MCH produced by these neurons would not be a neurohormone as in

fish. The great quantity of processes obsd. in the optic lobes and in the olfactive encephalic areas (particularly in the septum) is most probably related to behavioral and adaptive regulations controlled by the hypothalamus.

- Jee SH, Lee SY, Chiu HC, Chang CC, Chen TJ.
Effects of estrogen and estrogen receptor in normal human melanocytes. Biochem Biophys Res Commun 199(3):1407-12, 1994.
Abstract: Normal human melanocytes were cultured selectively with F12 culture medium supplemented with growth hormones, phorbol ester and 1% of fetal calf serum. The estrogen receptors were analyzed using hydroxylapatite-column assay with tritiated 17-beta-estradiol as the binding ligand. Phenol red- free medium was used when the changes in cell numbers, melanin content and tyrosinase were assessed after incubating with physiological concentration of 17-beta estradiol (10(-12) and 10(-9) M). It was found that the melanocytes contained both cytosol (5.42 +/- 1.11 fmol/mg protein) and nuclear (59.13 +/- 17.12 fmol/mg protein) estrogen receptor. In response to estradiol, the cell number increased but both the melanin content and the tyrosinase activity decreased in a dose related pattern. These data suggested the presence of estrogen receptor with biological function in normal human melanocytes.

- Hunt G, Todd C, Kyne S, Thody AJ.
ACTH stimulates melanogenesis in cultured human melanocytes. J Endocrinol 140(1):R1-3, 1994.
Abstract: While ACTH is known to induce skin pigmentation in man, its effects on cultured human melanocytes have not been investigated. Using a culture system free of artificial mitogens, we report for the first time that ACTH stimulates melanogenesis in cultured human melanocytes. While ACTH, alpha-MSH and the synthetic alpha-MSH analogue Nle4DPhe7 alpha-MSH all stimulate the activity of tyrosinase, the rate limiting enzyme in melanogenesis, and all produce a 50% increase in the melanin content of the cells at a concentration of 10(-8)-10(-7) mol/l, the shapes of the dose response curves differ: those for the MSH peptides are sigmoidal while those for ACTH are biphasic. In addition, human melanocytes are able to respond to concentrations of ACTH comparable with physiological plasma levels. We suggest that ACTH may be relatively more important than alpha-MSH as a pigmentary hormone in man and could have a physiological role in skin pigmentation.

- Hunt G, Todd C, Cresswell JE, Thody AJ.
Alpha-melanocyte stimulating hormone and its analogue Nle4DPhe7 alpha-MSH affect morphology, tyrosinase activity and melanogenesis in cultured human melanocytes. J Cell Sci 107(Pt 1):205-11, 1994.
Abstract: Although melanocyte stimulating hormone (MSH) peptides are known to stimulate pigmentation in man, previous reports suggest that human melanocytes are relatively unresponsive to these peptides in vitro. This may be related to the conditions under which the melanocytes were cultured. Thus, we have re-investigated the in vitro effects of MSH peptides using human melanocytes cultured in the absence of artificial mitogens. Human melanocytes were incubated with alpha-MSH or its potent analogue Nle4Dphe7 alpha-MSH for 3 days. After 18 hours, melanocyte morphology had evolved from mainly bipolar to dendritic in approximately 66% of cultures. Nle4DPhe7 alpha-MSH produced dose-related increases in both tyrosinase activity and melanin content although the degree of response was variable and tyrosinase activity was the relatively more responsive to the peptide. Similar results were obtained with alpha-MSH, but, although the effect on melanin content was similar to that of Nle4DPhe7 alpha-MSH, the effect on tyrosinase activity was less marked. The preliminary EC50 values for the actions of the MSH peptides suggest that they may be equipotent in their actions on human melanocytes. In addition, we have demonstrated that the common melanocyte mitogens 12-O-tetradecanoyl phorbol-13-acetate (TPA) and cholera toxin affect basal melanogenesis and modulate the effects of the MSH peptides. However, not all melanocyte cultures showed melanogenic responses to the MSH peptides. Ability to respond was unrelated to basal levels of tyrosinase activity or melanin content. In at least some cultures, morphological and melanogenic responses appear to be independent of one another.

- Pedeutour F; Szpirer C, Nahon JL.
Assignment of the human pro-melanin-concentrating hormone gene (PMCH) to chromosome 12q23-q24 and two variant genes (PMCH1 and PMCHL2) to chromosome 5p14 and 5q12-q13. Genomics 19(1):31-7, 1994.
Abstract: Melanin -concentrating hormone (MCH) is a peptide that has been isolated from salmon pituitary and rat hypothalamus. In mammals, pro-MCH (PMCH) encodes two putative peptides, named NEI and NGE, in addition to MCH. Those peptides are expressed predominantly in hypothalamus and display a broad array of functions in rat brain. We have previously mapped the PMCH locus on human chromosome 12q and rat chromosome 7. Genomic cloning has revealed the existence of two distinct MCH genes in human: one authentic and one variant. In this report, we describe Southern blotting analysis with DNA from a panel of somatic cell hybrids and demonstrate that the authentic human MCH (hMCH) gene is located as expected on chromosome 12, while the variant form of hMCH gene is located on chromosome 5. Direct chromosomal assignment of the authentic and variant hMCH genes was obtained by using fluorescence in situ hybridization on metaphase chromosomes. A strong signal was observed in 12q23-q24 with the authentic hMCH genomic DNA probe. Surprisingly, two signals were conspicuously found in 5p14 and 5q12-q13 with different variant hMCH genomic DNA probes. These loci were designated PMCHL1 and PMCHL2. Evidence of physiological and pathological data in rodents together with locus linkage analyses in human suggests that hMCH authentic and variant genes may be involved in human brain disorders.

-Sahm Ulrike G, Olivier George WJ, Branch Sarah K, Moss Stephen H, Pouton Colin W.

Influence of .alpha.-MSH terminal amino acids on binding affinity and biological activity in melanoma cells. Peptides, Tarrytown NY 15(3):441-6, 1994.
Abstract: The influence of the terminal amino acids of .alpha.-MSH on its biol. action in B16 murine melanoma cells has been systematically studied. Fragments of .alpha.-MSH lacking various sequences of terminal residues were synthesized by solid-phase peptide synthesis and their binding affinity to melanoma cells was measured using a radioreceptor assay. Biol. activity was detd. by measuring both tyrosinase activity and melanogenesis. The relative affinities and activities of the fragments generally followed the same pattern as found previously in other assay systems (frog and lizard bioassay and Cloudman S91 mouse melanoma), with the three amino acids at each terminal not being essential for binding and biol. activity, although the C-terminal amino acids 11-13 are more important than those in the N-terminus. The differences in biol. activity between the fragments can be explained by their relative binding affinities for the receptor.

- Siegrist W, Stutz S, Eberle AN.
Homologous and heterologous regulation of alpha-melanocyte- stimulating hormone receptors in human and mouse melanoma cell lines. Cancer Res 54(10):2604-10, 1994.
Abstract: Specific high-affinity receptors for alpha-melanocyte-stimulating hormone (alpha-MSH) are found in variable abundance on many melanoma cell lines. We have examined melanocortin peptides and other factors for their ability to regulate the number of MSH receptors in eleven human and two mouse melanoma cell lines. MSH induced up-regulation of its own receptors in three human cell lines and down-regulation in six human and two mouse melanoma cell lines. No regulation was observed in two human lines. Scatchard analysis revealed modulation of the number of receptors per cell without any change in affinity. The concentrations inducing half-maximal response for up- and down-regulation were 1.6 nM and 0.23 nM, respectively. ACTH1-17 and [Nle4,D-Phe7]-alpha-MSH were more potent, whereas ACTH1-24, desacetyl-alpha-MSH, and [Nle4]-alpha-MSH were less potent in receptor up-regulation as compared to alpha-MSH. Down-regulation but not up-regulation could be fully mimicked by Gs-protein activation and partially by elevation of cellular cAMP. Combination of different agents which increase cAMP was found to be counterregulatory. TPA and retinoic acid generally down-regulated MSH receptors but had no effect on HBL cells. Several protein kinase inhibitors increased MSH binding in B16 cells. MSH-induced receptor down-regulation and melanin synthesis were most effectively antagonized by selective inhibitors of cAMP-dependent protein kinase in these cells. Taken together, MSH receptors on melanoma cells are both positively and negatively regulated. Whereas cAMP-dependent protein kinase activation seems to be involved in down-regulation, the mechanism responsible for up-regulation remains to be elucidated.

4. Photobiology and photochemistry

- Aberdam E, Romero C, Ortonne JP.
Repeated UVB irradiations do not have the same potential to promote stimulation of melanogenesis in cultured normal human melanocytes. J Cell Sci 106(Pt 4):1015-22, 1993.
Abstract: The major stimulus for human melanin production is ultraviolet (UV) radiation. Little is known about the mechanisms underlying this response and the eventual enzyme regulation resulting from this activation. We treated normal human melanocytes in culture with daily UVB radiations. Cumulative increases in UVB doses resulted in proportional increases in tyrosinase activity over the first few days whereas an intermittent pattern of tyrosinase activation was observed after the fifth day of irradiation. This intermittent pattern consisted of latency periods where no melanogenic response was elicited despite exposure to UVB. Tyrosinase activity in cellular extracts increased shortly after an effective irradiation, peaked at 3 hours and thereafter decreased to below basal levels. Increased tyrosinase activity was associated with increased amounts of both the newly synthesized and mature forms of the enzyme. Decreased tyrosinase activity following an activation period was correlated with decreases in both the expression of tyrosinase mRNA and the amount of the newly synthesized form of the enzyme present in the melanocytes 24 hours after six irradiations. This particular pattern of stimulation of tyrosinase was not observed in S-91 murine melanoma cells after repeated UVB irradiations. Taken together these results may suggest a photo-protective mechanism developed by irradiated normal human melanocytes.

- Braida D, Dubief C, Lang G.
Photoageing of hair fiber and photoprotection. Skin Pharmacol 7(1-2):73-7, 1994.
Abstract: Examination by transmission electron microscopy of hair exposed to sunlight has revealed important damage. Findings indicate an alteration of various cell components. Damage occurs in the cuticle and leads to its loss. Separation of macrofibrils and destruction of melanin pigments result in cortex damage. Some of the chemical and physical changes which occur in hair exposed to sunlight were studied: formation of carbonyl groups, cystine destruction, modification of the proteins obtained by reduction of the disulfide bonds, losses in mechanical strength and discoloration are discussed. Effects of UV and visible radiations were studied. All of them cause modification in hair properties. Experiments with artificial light sources were carried out to reproduce these alterations. The role of water during exposure was studied. In particular, discoloration of brown hair is largely affected by it. We showed that Xenon lamps were useful to follow the photooxidation of hair and simulate natural alterations. The properties of some protective materials were examined.

-de Leeuw SM, Janssen S, Simons JW, Lohman PHM, Vermeer BJ, Schothorst AA.
The UV action spectra for the clone-forming ability of cultured human melanocytes and keratinocytes. Photochem. Photobiol. 59:430-436, 1994.
Abstract: Melanocytes (skin type 2) and keratinocytes were irradiated with UV light of 254, 297, 302, 312 and 365 nm and the survival was measured. Clone-forming ability was chosen as the parameter for cell survival. Melanocytes were found to be less sensitive to UV light than keratinocytes (a difference of a factor 1.22-1.92 for the UV-C and UV-B wavelengths 254, 297, 301 and 312 nm) and a factor 6.71 for the UV-A wavelength (365 nm). Because melanin does not appear to protect against the induction of pyrimidine dimers the difference between melanocytes and keratinocytes in the UV-C and UV-B region could not be explained by the presence of melanin in the melanocytes. The relatively small difference can be explained by the longer cell cycle of melanocytes, which provides more time for the melanocytes to repair UV damage. In the UV-A region the difference between melanocytes and keratinocytes was much larger, suggesting that besides the longer cell cycle some addnl. factors must be involved in protection against UV-A light.

- Griffiths GE, Voorhees JJ.
Topical retinoic acid for photoaging: clinical response and underlying mechanisms. Skin Pharmacol 6 Suppl 1:70-7, 1993.
Abstract: Photoaging is primarily composed of wrinkling, mottled hyperpigmentation and a tactile roughness of the skin, all three of these parameters improve following use of topical retinoids. It appears that smoothening of the skin results from a combination of epidermal changes including thickening, stratum corneum compaction and glycosaminoglycan deposition. Lightening of actinic lentigines and mottled hyperpigmentation correlates with a reduction in epidermal melanin content maybe resulting from inhibition of tyrosinase activity. Effacement of wrinkling in mice correlates with new collagen synthesis, and there is evidence that this is also the case in humans. An irritant dermatitis is a feature of retinoid-treated skin but this diminishes in severity during treatment despite continued improvement in photoaging. Thus it is unlikely that irritation per se is responsible for clinical improvement.

- Kimura T, Doi K.
Responses of the skin over the dorsum to sunlight in hairless descendants of Mexican hairless dogs. Am J Vet Res 55(2):199-203, 1994.
Abstract: Responses of the skin over the dorsum to solar UV irradiation (2 hours/d for 6 consecutive days) were investigated in hairless descendants of Mexican hairless dogs. Assessment of skin color changes, using a spectrophotometer, indicated that luminance values began to decrease from the third day of UV irradiation, reached the minimal value at 3 weeks, and almost recovered 12 weeks after completion of UV irradiation. The number of the dihydroxyphenylalanine-positive melanocytes increased significantly ($P < 0.01$) from the third day of UV irradiation, reached its maximal value at 2 weeks, and recovered to normal at 12 weeks after completion of UV irradiation. On the second day of UV irradiation, the epidermis became focally thick, with disarrangement of component cells that had degenerative changes. In addition, a few so-called sunburn cells with pyknotic nuclei were seen in the epidermis. On the third day of UV irradiation, apparent suntan reaction developed, and a large number of epithelial cells in the epidermis were heavily pigmented with melanin granules. At 12 weeks after completion of UV irradiation, the epidermis appeared almost normal. On the other hand, significant changes were not detected in the dermis throughout the study.

- Noz KC, Roza L, Bergman W, Daroudi F, Schothorst AA.
UV induction of cyclobutane thymine dimers in the DNA of cultured melanocytes from foreskin, common melanocytic nevi and dysplastic nevi Photochem. Photobiol. 59:534-540, 1994.
Abstract: The authors compared the induction of cyclobutane thymine dimers after exposure to 302 nm UV in foreskin-derived melanocytes and melanocytes from nevocellular nevi, as well as in melanocytes cultured from dysplastic nevi, precursor lesions of melanoma, derived from four, three and four individuals, resp. Cyclobutane thymine dimers were quantified in situ by means of an immunofluorescence assay with a specific monoclonal antibody. A method was developed to compare sep. performed expts. in a standardized manner. For melanocytes from each source, the authors demonstrated a linear relationship between UV dose and immunofluorescence. In nevocellular and dysplastic nevi, two subpopulations could be detected, distinguished by their nuclear size. Large nucleated nevocellular nevus cells were most susceptible to the induction of thymine dimers (49% higher induction compared to induction in foreskin melanocytes), while in normal-sized nuclei of these nevus cells the same induction of thymine dimers was found as in nuclei from foreskin melanocytes. In contrast, large nucleated dysplastic nevus melanocytes did not differ from the foreskin melanocytes, while normal-sized nuclei of dysplastic nevus cells showed a lower induction (32% lower induction than in foreskin melanocytes), while normal-sized nuclei of dysplastic nevus cells showed a lower induction (32% lower induction than in foreskin melanocytes).

- Takiwaki H, Serup J.
Measurement of color parameters of psoriatic plaques by narrow-band reflectance spectrophotometry and tristimulus colorimetry. Skin Pharmacol 7(3):145-50, 1994.
Abstract: Color parameters were measured on 50 psoriatic plaques in 10 patients, after scoring the amount of scales on them by inspection, with a narrow-band reflectance spectrophotometer (erythema/ melanin index expression) and tristimulus colorimeter (CIE L a b expression). Both erythema index a (redness) were highest in the group of erythematous plaque with little scale (twice as high as in

controls) and decreased significantly as the plaques were covered with thicker scale, while L (brightness) changed in just the opposite fashion of a . These portable 'color indicators' can be utilized to express the appearance of psoriatic plaques quantitatively, especially the extent of erythema and the amount of the scale on it.

5. Neuromelanins

Comments by Dr M. d'Ischia:

The concept of neuromelanin as a mixed-type melanin is consolidated by an interesting paper by **Odh et al.** (J. Neurochem. 62,2030-2036, 1994), which integrates and extends previous studies by the same group (Carstam et al., Biochim. Biophys. Acta 1097,152-160, 1991). Using a degradative approach based on permanganate oxidation of isolated human neuromelanin and model synthetic pigments, prepared by co-oxidation of dopamine and cysteinyldopamine, these authors conclude that neuromelanin is mainly made up of benzothiazine and indole-type units in approximately equal amounts. The known limitations inherent in the method do not permit to clearly discriminate between an authentic co-polymer of dopamine and cysteinyldopamine, in which indole and benzothiazine units are intimately mixed up in the pigment backbone, and a mixture of chemically distinct, homogeneous polymers. Yet, the fact remains that neuromelanin shows much like a heterogeneous entity made up to a considerable degree of cysteinyldopamine-derived units. It is tempting to speculate that the presence of negatively-charged carboxyl groups due to cysteinyldopamine may partly account for the pronounced cation-exchange properties of neuromelanin.
In this connection, a systematic analysis of trace metals, carried out by **Zecca and coworkers** (J. Neurochem. 62,1097-1101, 1994) using radiochemical neutron activation analysis, has definitively confirmed the presence of significant amounts of iron and, to a lower extent, zinc in purified human neuromelanin as well as in the substantia nigra and putamen.
In the biomedical context, mention goes to a paper by **Takahashi et al.** (Neurology, 44,437-441, 1994) reporting an interesting example of familial juvenile parkinsonism. Pathological changes at autopsy included a substantial loss of melanin-containing neurons in the substantia nigra pars compacta and locus coeruleus, but no formation of Lewy bodies, the characteristic hallmark of idiopathic Parkinson's disease, was detected. Up till now, the significance of genetics in Parkinson's disease has been rather controversial, and it has been proposed that the idiopathic disease is the result of environmental factors acting on a genetic background. The observation by Takahashi et al. would apparently argue in favour of a genetic basis of the disease, at least for what concerns the juvenile variant. It also underscores the many complexities underlying the biochemical pathology of Parkinson's disease, including the role of neuromelanin versus Lewy bodies as the key to understanding the origin of the disorder.

- Marinho CR, Manso CF.
O2 generation during neuromelanin synthesis. The action of manganese. Acta Med Port 6(11):547-54, 1993.
Abstract: The oxygen uptake during the reaction of dopamine autoxidation was studied and it was found that it may occur through two distinct oxidative pathways. One involves the univalent reduction of oxygen, forming the superoxide radical, the other involves a bivalent reduction which generates hydrogen peroxide. The detection of the univalent reaction was followed by the study of oxygen consumption, in the presence of cytochrome c. The detection of the bivalent reaction was made in the presence of catalase. It was found that manganese increases the oxygen uptake of the reaction, by increasing the production of both oxygen active forms. It seems probable that the neurotoxicity of this metal may be related with the increased production of activated oxygen in dopaminergic neurons, which undergo an oxidative stress.

-Odh G, Carstam R, Paulson J, Wittbjer A, Rosengren E, Rorsman H.
Neuromelanin of the human substantia nigra: a mixed-type melanin. J Neurochem 62(5):2030-6, 1994.

- Stein ME, Loberant N, Kessel I, Kuten A.
Melanocytic schwannoma of the spinal cord: a case report. East Afr Med J 70(9):597-9, 1993.
Abstract: Melanocytic schwannoma of the spinal cord is a tumour composed of Schwann cells capable of producing intracellular melanin . We present a case report of a 25-year-old male with melanocytic schwannoma of the 5th lumbar spinal cord root; the patient is alive and well 48 months after neurosurgical removal of the tumour. A review of the literature revealed 14 similar cases. These tumours have a local recurrence rate of up to 14%, and have a metastatic potential.

-Takahashi H, Ohama E, Suzuki S, Horikawa Y, Ishikawa A, Morita T, Tsuji S, Ikuta F.
Familial juvenile parkinsonism: clinical and pathologic study in a family. Neurology, 44(3 Pt 1):437-41, 1994.

- Zecca L, Pietra R, Goj C, Mecacci C, Radice D, Sabbioni E.
Iron and other metals in neuromelanin, substantia nigra, and putamen of human brain. J Neurochem 62(3):1097-1101, 1994.

6. Genetics, molecular biology

- Bultman SJ, Klebig ML, Michaud EJ, Sweet HO, Davisson MT, Woychik RP.
Molecular analysis of reverse mutations from nonagouti (a) to black- and-tan (a(t)) and white-bellied agouti (Aw) reveals alternative forms of agouti transcripts. Genes Dev 8(4):481-90, 1994.
Abstract: The agouti gene regulates the differential production of eumelanin (black or brown) and phaeomelanin (yellow) pigment granules by melanocytes in the hair follicles of mice. The original nonagouti (a) allele, which confers a predominantly black coat color, has been shown to revert to two other more dominant agouti alleles, black-and-tan (a(t)) and white-bellied agouti (Aw), with an exceptionally high frequency. The a(t) and Aw alleles confer phenotypes in which the pigmentation is not uniformly distributed over the dorsal and ventral surfaces of the animal; in both cases the ventral surface of the animal is markedly lighter than the dorsal surface due to an increase in phaeomelanin production. To understand the unusually high reversion rate of a to a(t) or Aw, and to decipher the molecular events associated with the different pigmentation patterns associated with these three agouti alleles, we have characterized a, a(t) and Aw at the molecular level. Here, we report that insertions of 11, 6, and 0.6 kb are present at precisely the same position in the first intron of the agouti gene in a, a(t), and Aw, respectively. The a insertion consists of a 5.5-kb VL30 element that has incorporated 5.5 kb of additional sequence internally; this internal sequence is flanked by 526-bp direct repeats. The a(t) allele contains only the VL30 element and a single, internal 526-bp repeat. The Aw allele has only a solo VL30 LTR. Based on the comparison of the structure of the a(t) and Aw insertions, we propose that reverse mutations occur by excision of inserted sequences in a through homologous recombination, utilizing either the 526-bp direct repeats to generate a(t) or the VL30 LTRs to generate Aw. Moreover, the analysis of these three alleles has allowed us to identify additional exons of the agouti gene that give rise to alternatively processed forms of agouti mRNA. We demonstrate that the distinct insertions in a, a(t) and Aw cause pigmentation differences by selectively inactivating the expression of different forms of agouti transcripts.

- Han K, Hong J, Lim HC, Kim CH, Park Y, Cho JM.
Tyrosinase production in recombinant E. coli containing trp promoter and ubiquitin sequence. Ann N Y Acad Sci 721:30-42, 1994.
Abstract: We have successfully expressed the active tyrosinase of Streptomyces antibioticus in Escherichia coli under the control of the trp promoter by fusing the sequence to the ORF438 gene. Because our attempt to connect the polycistronic gene of ORF438 and tyrosinase directly to the trp promoter of E. coli resulted in the expression of functionally inactive tyrosinase, we decided to fuse the COOH-terminus of ubiquitin sequence to the NH2-terminus of ORF438. Ubiquitin fusion has been shown to augment the yield of cloned gene products in E. coli by increasing the stability or translational efficiency of the fusion proteins. As a result, E. coli transformants harboring a plasmid pTRUBF that contains the ubiquitin-fused ORF438 and the tyrosinase gene produced the strong black pigment of melanin . About 300 units of tyrosinase per liter of batch culture were detected when cultivated in M9 medium containing casamino acids, L-tyrosine, and copper supplements. The black pigment, however, was not seen when grown in LB medium, suggesting that the trp promoter is well regulated. When recombinant E. coli cells grown in LB medium were transferred to a tryptophan-deficient minimal medium with phenol, we observed that phenol was removed from the solution, and the color of the medium turned black. This is due to the fact that the tyrosinase has polyphenol oxidase properties. We expect to use this recombinant E. coli for the waste treatment of phenolic compounds.

- Kedda MA, Stevens G, Manga P, Viljoen C, Jenkins T, Ramsay M.
The tyrosinase-positive oculocutaneous albinism gene shows locus homogeneity on chromosome 15q11-q13 and evidence of multiple mutations in southern African negroids. Am J Hum Genet 54(6):1078-84, 1994.
Abstract: Tyrosinase-positive oculocutaneous albinism (ty-pos OCA) is an autosomal recessive disorder of the melanin pigmentary system. South African ty-pos OCA individuals occur with two distinct phenotypes, with or without darkly pigmented patches (ephelides, or dendritic freckles) on exposed areas of the skin. These phenotypes are concordant within families, suggesting that there may be more than one mutation at the ty-pos OCA locus. Linkage studies carried out in 41 families have shown linkage between markers in the Prader-Willi/Angelman syndrome (PWS/AS) region on chromosome 15q11-q13 and ty-pos OCA. Analysis showed no obligatory crossovers between the alleles at the D15S12 locus and ty-pos OCA, suggesting that the D15S12 locus is very close to or part of the disease locus, which is postulated to be the human homologue, P, of the mouse pink-eyed dilution gene, p. Unlike caucasoid "ty-pos OCA" individuals, negroid ty-pos OCA individuals do not show any evidence of locus heterogeneity. Studies of allelic association between the polymorphic alleles detected at the D15S12 locus and ephelus status suggest that there was a single major mutation giving rise to ty-pos OCA without ephelides. There may, however, be two major mutations causing ty-pos OCA with ephelides, one associated with D15S12 allele 1 and the other associated with D15S12 allele 2. The two loci, GABRA5 and D15S24, flanking D15S12, are both hypervariable, and many different haplotypes were observed with the alleles at the three loci on both ty-pos OCA-associated chromosomes and "normal" chromosomes.

- Lee ST, Nicholls RD, Bundey S, Laxova R, Musarella M, Spritz RA.
Mutations of the P gene in oculocutaneous albinism, ocular albinism, and Prader-Willi syndrome plus albinism. N Engl J Med 330(8):529-34, 1994.
Abstract: BACKGROUND. Type II (tyrosinase-positive) oculocutaneous albinism is an autosomal recessive disorder that has recently

been mapped to chromosome segment 15q11-q13. The frequency of this disorder is greatly increased in patients with Prader-Willi or Angelman syndrome, both of which involve deletions of chromosome 15q. The P protein is a transmembrane polypeptide that may transport small molecules such as tyrosine, the precursor of melanin . The P gene is located in chromosome segment 15q11-q13. METHODS. We studied the tyrosinase and P genes in three patients with type II oculocutaneous albinism, one of whom also had Prader-Willi syndrome, and in one patient with a milder syndrome known as autosomal recessive ocular albinism. Individual exons of these genes were amplified from the DNA of each patient by the polymerase chain reaction and screened for mutations by simultaneous analyses of single-stranded conformation polymorphisms and heteroduplexes and subsequent DNA sequencing. RESULTS. Mutations of the P gene were identified in all four patients. These included one frame shift, three missense mutations that result in amino acid substitutions, and one mutation that affects RNA splicing. The patient with Prader-Willi syndrome plus albinism had a typical deletion of the paternal chromosome 15, rendering him hemizygous for a maternally inherited mutant allele of the P gene. The child with ocular albinism was heterozygous for two different mutations in the P gene. CONCLUSIONS. Abnormalities of the P gene are associated with a wide range of clinical phenotypes, including type II oculocutaneous albinism, albinism associated with the Prader-Willi syndrome, and at least some cases of autosomal recessive ocular albinism.

- Ponnazhagan S, Hou L, Kwon BS.
Structural organization of the human tyrosinase gene and sequence analysis and characterization of its promoter region. J Invest Dermatol 102(5):744-8, 1994.
Abstract: Tyrosinase is the principal enzyme in the biosynthesis of melanin . The expression of tyrosinase is tissue-specific and appears to be regulated by various hormonal and environmental factors. Elucidation of the genomic structure and molecular basis of control of tyrosinase gene expression will greatly enhance our understanding of the regulation of human pigmentation. To this end, we have isolated and performed restriction mapping of recombinant cosmid and lambda phage clones containing the human tyrosinase gene, sequenced a 2.2-kilobase (kb) region of its promoter, and determined the potential regions regulating the tyrosinase gene expression in transient-expression system. The human tyrosinase gene is comprised of five exons and four introns. Based on our restriction mapping studies, the gene spans a distance of over 65-kb on chromosome 11 (q14-->q21). We constructed a series of plasmids (pHTY-CAT) that contain 5' sequential deletions of the human tyrosinase 5' flanking sequence fused to the reporter gene, chloramphenicol acetyltransferase (CAT). The plasmids were used to locate promoter regions that are potential regulators of tyrosinase gene expression in a transient expression system using melanoma cell lines. In human melanoma cells, the plasmid construct with a -2020 base pair (bp) promoter yielded the highest CAT activity. When the deletions reached -1739 bp, the CAT activity was dramatically reduced, indicating that important enhancer elements for transcription control are present between -1739 and -2020 bp. Further deletions up to -550 bp also resulted in dramatic decreases of CAT activity. However, when the deletion included -550 bp of the 5' flanking sequence, there was 26 percent of the CAT activity compared to that of the -2020 bp promoter. Deletions beyond -550 bp also showed markedly decreased CAT activity. Based on our data, we suggest that human tyrosinase gene expression is governed by both tissue-specific and multiple regulatory elements.

- Schmidt A, Beermann F.
Molecular basis of dark-eyed albinism in the mouse. Proc Natl Acad Sci U S A 91(11):4756-60, 1994.
Abstract: Dark-eyed albino (C44H) is a recessive allele at the mouse albino (c) locus, which encodes tyrosinase (monophenol,L-dopa:oxygen oxidoreductase, EC 1.14.18.1), the key enzyme in melanin synthesis. Similar to type IB oculocutaneous albinism in humans, overall production of pigment is greatly reduced in dark-eyed albino mice and obvious only in the eyes. We have studied the molecular basis of the c44H mutation and show that expression of the tyrosinase gene is not affected. After sequencing tyrosinase cDNA isolated from c44H/c44H homozygotes, we uncovered a single base alteration from wild type leading to a serine-to-isoleucine exchange. The importance of this mutation was demonstrated by generating transgenic mice containing a mutated tyrosinase minigene. This showed that the single base change was sufficient to severely depress pigment production in transgenic mice. We therefore conclude that the point mutation is responsible and sufficient to generate the dark-eyed albino phenotype.

- Vidal-Cros A, Viviani F, Labesse G, Boccara M, Gaudry M.
Polyhydroxynaphthalene reductase involved in melanin biosynthesis in Magnaporthe grisea. Purification, cDNA cloning and sequencing. Eur J Biochem 219(3):985-92, 1994.
Abstract: During the biosynthesis of fungal melanin , tetrahydroxynaphthalene reductase catalyzes the NADPH-dependent reduction of 1,3,6,8-tetrahydroxynaphthalene (T4HN) into (+)-scytalone and 1,3,8-trihydroxynaphthalene into (-)-vermelone. The enzyme from Magnaporthe grisea, the fungus responsible for rice blast disease, has been purified to homogeneity. It is a tetramer of four identical 30-kDa subunits. A full-length cDNA clone of about 1 kb encoding T4HN reductase has been isolated from a cDNA library constructed in the lambda ZAP II vector and characterized. The clone contains a 846-bp open reading frame. Translation of the DNA sequence gave a 282-residue amino acid sequence with a calculated molecular mass of 29.9 kDa. Sequences corresponding to the amino-terminal part and three internal proteolytic peptides were present in the translated sequence. T4HN reductase exhibits characteristics of the short-chain alcohol dehydrogenase family. The reductase shares 56% identity with a putative ketoreductase involved in aflatoxin biosynthesis in Aspergillus parasiticus.

7. Tyrosinase, TRP1, TRP2 and other enzymes

Comments by Prof. J.C. Garcia-Borron:

As a result of the increasing awareness of the important role of the tyrosinase related proteins and other non-tyrosinase factors on the regulation of melanogenesis, the number of papers dealing with the structure and function of these proteins is growing steadily. The interesting paper by **Hara et al** (J. Invest Dermatol, 102:495-500) shows that the regulation by UV radiation of tyrosinase and TRP-1 is similar, and, moreover, that
TRP-1 is necessary for melanogenesis. However, the actual role of the protein ios still to be determined.

As far as TRP-2 is concerned, **Cassady and Sturm** (Gene 143:295-298) have cloned and sequenced the human gene coding for the enzyme. The availability of a specific probe made it possible to demonstrate that its level of expression in human melanoma cells are high. These findings have important consequences. The high degree of sequence conservation and the presence of the protein in mouse and human cells suggest that its role on pigment cell biology is prominent. Its presence in melanoma cells might suggest novel chemotherapeutic approaches to melanoma treatment.

On the other hand, work by **Palumbo et al.** (Biochem J 299:839-844) has characterized a novel enzyme activity from the ink of Sepia, that converts dopachrome into DHI. This raises the question as to whether this enzyme is more similar to the insect dopachrome converting activity described by Sugumaran's group than to mammalian dopachrome tautomerase. In any case, the findings by the group of Naples prove that the occurrence of dopachrome converting enzymes is a widespread, maybe even general, phenomenon.

- Cassady JL, Sturm RA.
Sequence of the human dopachrome tautomerase-encoding TRP-2 cDNA. Gene 143(2):295-8, 1994.

-Hara H, Lee MH, Chen H, Luo D, Jimbow K.
Role of gene expression and protein synthesis of tyrosinase, TRP-1, lamp-1, and CD63 in UVB-induced melanogenesis in human melanomas. J Invest Dermatol, 102(4):495-500, 1994.

-Le Poole IC, van den Wijngaard RM, Smit NP, Oosting J, Westerhof W, Pavel S.
Catechol-O-methyltransferase in vitiligo. Arch Dermatol Res 286(2):81-6, 1994.
Abstract: Catechol-O-methyltransferase (COMT) is involved in the metabolism of neurotransmitters such as epinephrine, norepinephrine and dopamine. For melanocytes, the enzyme is of particular importance in preventing the formation of toxic o-quinones during melanin synthesis. It has been suggested that COMT plays a regulatory role in melanin synthesis. Indeed, when the melanin precursor molecule DHI(2C) is methylated by COMT it is no longer available for incorporation into melanin . Auto-destruction by intermediates of melanin metabolism has been implicated in the aetiology of vitiligo. Therefore enzyme activities in vitiligo patients and in healthy controls were compared. Systemic COMT activities were measured using red blood cells (RBC) as starting material. However, as local alterations in COMT activity may be specifically involved in vitiligo, the enzyme activity was also measured in epidermal homogenates. Finally, to ascribe epidermal COMT activity to the responsible cell type(s), enzyme activity was measured in cultured vitiligo non-lesional melanocytes and melanocytes from healthy controls as well as in cultured keratinocytes from lesional skin and in purified keratinocytes from control skin. It was found that epidermal homogenates from vitiligo patients expressed higher levels of COMT activity than homogenates from healthy controls. Such differences were not found at the systemic level (i.e. in RBC) nor could they be explained by measurements on separately cultured epidermal cell types, indicating that the COMT activity was induced at the tissue level by extracellular factors.

-Palumbo A, d'Ischia M, Misuraca G, De Martino L, Prota G.
A new dopachrome-rearranging enzyme from the ejected ink of the cuttlefish Sepia officinalis. Biochem J 299(Pt 3):839-44, 1994.

8. Melanoma and other pigmented tumours

Comments by Dr N. Smit:

Several experimental approaches have been described which may be of interest in melanoma investigations and possibly for future application in clinical treatment.
As a chemotherapeutic agent the 9-nitro-derivative of a plant alkaloid camptothecin (9NC) has been tested on "normal" and malignant melanocytes in vitro. It was demonstrated that the melanoma cells treated with 9NC were arrested in S-phase and subsequently died whereas treated melanocytes accumulated at the S/G_2 boundary with only small numbers of cells dying (**Pantazis et al**).
The method of using in vitro cultured tumor-infiltrating lymphocytes (TIL) for treatment of melanoma tumors was improved by injecting tumor cells in combination with rIL-6 in a collagen matrix in a mice model. This method was applied in order to achieve increased local concentrations and prolonged release of IL-6 which resulted in isolation of TIL with enhanced antitumor activity as compared to controls

injected with rIL-6 or matrix alone (**Marcus et al**).
Human melanoma spheroids have been used as a model to test the anti-tumor effects of interferons in a study by **Görlach et al**. In this culture system the high anti-proliferative sensitivity as found in melanoma monolayer cultures is not observed showing that the spheroids may serve as a better tumor model.
Rodeck et al describe that melanoma cell lines showed various degrees of resistance to the inhibition of DNA synthesis by exogeneous transforming growth factor-ß as compared to normal melanocytes. It is speculated that the paracrine effects of TGF-ß may outweigh the growth inhibitory effect of this growth factor. In this respect the work described by **Saiki et al** may offer a nice example of such favorable effects since it was shown that the production of TGF-ß1 by the tumor cells may be responsible for increased production of fibronectin by fibroblasts which was suggested as a possible reason for the higher invasive and migratory activity of the highly metastatic melanoma cell line used in this study.
The work of **Ruck et al** shows the adaptation of several bladder tumor cell lines as well as a melanoma cell line to serum free growth conditions. Autocrine stimulation is suggested as mechanism of survival for the cells. On the other hand this approach, like cell culture in general may lead to a selection of a subpopulation of cells. The work of **Stackpole et al** could be a demonstration of such selection of a highly malignant phenotype under extreme circumstances by subjecting melanoma cells to severe hypoxia.

- Alena F, Iwashina T, Gili A, Jimbow K.
Selective in vivo accumulation of N-acetyl-4-S-cysteaminylphenol in B16F10 murine melanoma and enhancement of its in vitro and in vivo antimelanoma effect by combination of buthionine sulfoximine. Cancer Res 54(10):2661-6, 1994.
Abstract: In order to develop a new chemotherapeutic agent based on exploitation of the specific metabolic pathway of malignant melanoma, a phenolic thioether, N-acetyl-4-S-cysteaminylphenol (NA-CAP), the substrate of melanin -forming enzyme, tyrosinase was developed. Our previous in vivo studies have clearly shown that this compound has a significant and selective melanocytotoxicity and antimelanoma effect. This study further examined the specificity of the antimelanoma effect of NA-CAP through the study of biodistribution and accumulation of NA-CAP in B16F10 melanoma-bearing mice. We also tested the antimelanoma effect of NA-CAP by combination treatment with buthionine sulfoximine on the growth of in vitro culture cells and in vivo B16F10 melanoma lung colonies. We found a selective accumulation of 14C-labeled NA-CAP into s.c. transplants and lung colonies of melanoma grown in C57BL mice. This accumulation was mediated by selective covalent binding of NA-CAP to the melanoma tissues. The combination of NA-CAP and buthionine sulfoximine significantly increased the chemosensitivity of B16F10 melanoma cells in vitro and reduced the number of in vivo melanoma lung colonies. We conclude that NA-CAP acts as an alkylating agent to melanoma tissue and that the combination of buthionine sulfoximine enhances the therapeutic index of this potent melanoma-specific drug through the depletion of tissue glutathione.

- Anstey A, Cerio R, Ramnarain N, Orchard G, Smith N, Jones EW.
Desmoplastic malignant melanoma. An immunocytochemical study of 25 cases. Am J Dermatopathol 16(1):14-22, 1994.
Abstract: Using a panel of seven cell markers, we studied the value of immunocytochemical labelling in the histological diagnosis of desmoplastic malignant melanoma. Sections from routine formalin-fixed tissue of 45 surgical specimens were obtained from 25 cases of malignant melanoma that showed well-marked desmoplastic or neurotropic features. Routinely stained sections (Haematoxylin- and -eosin and melanin stains) were compared with the following panel of seven antibodies: S-100, neuron-specific enolase (NSE), vimentin, factor XIIIa (FXIIIa), desmin and the newer, supposedly more specific antimelanoma antibodies HMB45 and NKIC3. S-100 and NSE were the most sensitive antibodies for desmoplastic malignant melanoma with strong labelling of spindle cells in most cases. In contrast, results for NKIC3 were more variable; results were negative in nearly half the tumours, but strong labelling was seen in six cases (27%). Positive labelling for HMB45 was noted in five tumours (22%); it was mostly confined to small groups of cells in the superficial part of the lesions. Tumour spindle cells were negative for FXIIIa in all cases; there was no increase in the number of positive dermal dendritic cells compared to conventional and spindle cell melanoma. All tumours were desmin-negative, but most were vimentin-positive. Our findings indicate that immunocytochemistry is of less value in the diagnosis of desmoplastic malignant melanoma that it is with other types of malignant melanoma. However, positive or negative labelling for S-100 protein and NSE is useful for suggesting or excluding a diagnosis of desmoplastic malignant melanoma; neither marker is specific and, in particular, positive labelling is also found in most neurofibromas and benign cellular naevi.

- Ball NJ, Golic NE.
Melanocytic nevi with focal atypical epithelioid cell components: a review of seventy-three cases. J Am Acad Dermatol 30(5 Pt 1):724-9, 1994.
Abstract: BACKGROUND: We report a variant of melanocytic nevus that may be confused with melanoma. OBJECTIVE: The purpose of this study is to describe the clinical, histologic, and biologic features of nevi with focal atypical epithelioid cell components (clonal nevi). METHODS: Seventy-three cases were retrieved by reviewing lesions previously diagnosed as clonal, combined, deep penetrating, and inverted type-A nevi. Histologic features were assessed and referring physicians received a questionnaire about the presentation and outcome of each case. RESULTS: Histologically, all cases had a biphasic pattern characterized by an ordinary nevus that contained a darkly pigmented collection of large distinct epithelioid melanocytes in the superficial dermis. Immunostains identified mutant p53 proteins in 50% of dermal clones (9 of 18) but not in ordinary nevus cells adjacent to the clones. We are not aware of any patient

developing a malignant melanoma (mean follow-up 24.5 months), including 41 cases that were initially incompletely excised. CONCLUSION: Clonal nevi are a distinct variant of melanocytic nevi and should be distinguished from malignant melanoma arising in a preexisting nevus.

-Barth RF, Matalka KZ, Bailey MQ, Staubus AE, Soloway AH, Moeschberger ML, Coderre JA, Rofstad EK.
A nude rat model for neutron capture therapy of human intracerebral melanoma. Int J Radiat Oncol Biol Phys 28(5):1079-88, 1994.
Abstract: PURPOSE: The present study was carried out to determine the efficacy of Boron Neutron Capture Therapy (BNCT) for intracerebral melanoma using nude rats, the human melanoma cell line MRA 27, and boronophenylalanine as the capture agent. METHODS AND MATERIALS: Pharmacokinetic and tissue distribution studies: MRA 27 cells (2 x 10(5)) were implanted intracerebrally, and 30 days later, 120 mg of 10B-L-BPA were injected intraperitoneally into nude rats. Therapy experiments: Thirty days following implantation, tumor bearing rats were irradiated at the Brookhaven Medical Research Reactor. RESULTS: Pharmacokinetic experiments: Six hours following administration of BPA, tumor, blood, and normal brain boron-10 levels were 23.7, 9.4, and 8.4 micrograms/g respectively. Therapy experiments: Median survival time of untreated rats was 44 days compared to 76 days and 93 days for those receiving physical doses of 2.73 Gy and 3.64 Gy, respectively. Rats that had received both 10B-BPA and physical doses of 1.82, 2.73, or 3.64 Gy had median survival times of 170, 182, and 262 days, respectively. Forty percent of rats that had received the highest tumor dose (10.1 Gy) survived for > 300 days and in a replicate experiment 21% of the rats were longterm survivors (> 220 days). Animals that received 12 Gy in a single dose or 18 Gy fractionated (2 Gy x 9) of gamma photons from a 137Cs source had median survival times of 86 and 79 days, respectively, compared to 47 days for untreated animals. Histopathologic examination of the brains of longterm surviving rats, euthanized at 8 or 16 months following BNCT, showed no residual tumor, but dense accumulations of melanin laden macrophages and minimal gliosis were observed.
CONCLUSION: Significant prolongations in median survival time were noted in nude rats with intracerebral human melanoma that had received BNCT thereby suggesting therapeutic efficacy. Large animal studies should be carried out to further assess BNCT of intracerebral melanoma before any human trials are contemplated.

-Cole DJ, Taubenberger JK, Pockaj BA, Yannelli JR, Carter C, Carrasquillo J, Leitman S, Steinberg SM, Rosenberg SA, Yang YC.
Histopathological analysis of metastatic melanoma deposits in patients receiving adoptive immunotherapy with tumor-infiltrating lymphocytes. Cancer Immunol Immunother 38(5):299-303, 1994.
Abstract: Tumor-infiltrating lymphocytes (TIL) from a wide range of human and murine tumors can be expanded in vitro using interleukin-2 (IL-2). These TIL are cytolytic T lymphocytes with in vivo and in vitro antitumor activity in mice and in humans. TIL from human melanoma can recognize autologous tumor in an MHC-restricted fashion, localize in vivo after 111In labeling, and mediate regression of large metastatic deposits. Although studied extensively in vitro, less is known in vivo about TIL activity associated with tumor regression. This study was undertaken, in association with a study of TIL localization, to investigate mechanisms of TIL action by evaluating histopathological changes that occur at the tumor site during TIL administration. A total of 106 pre- and post-treatment pathological specimens from 25 patients enrolled in phase II TIL treatment and 111In-TIL imaging protocols were examined blindly by a single pathologist. Histological subtype, lymphocytic infiltration, melanin content, vascularity, and necrosis were documented for each tumor specimen. Average baseline and post-treatment parameters were compared. Any significant changes were evaluated for correlation with clinical response and 111In-TIL localization to tumor. Melanin content and vascularity of the tumor did not change as a result of therapy or correlate with either response or TIL localization. However, both increased lymphocytic infiltration and tumor necrosis were present after TIL administration ($P = 0.044$ and 0.032 respectively). Furthermore, increases in lymphocytic infiltration correlated with tumor imaging using 111In-TIL, and with the percentage of 111In-labeled injectate present per gram of tumor specimen ($P = 0.036$ and 0.0041 respectively). This suggests that TIL either account for the increased lymphocytes directly, or localize to tumor and recruit endogenous lymphocytes. We were unable to demonstrate any pretreatment histopathological predictors of response or variables that significantly correlated with subsequent clinical response, although peak and average values of necrosis were higher in responding patients compared to non-responding patients.

- Gazit D, Daniels TE.
Oral melanocytic lesions: differences in expression of HMB-45 and S-100 antigens in round and spindle cells of malignant and benign lesions. J Oral Pathol Med 23(2):60-4, 1994.
Abstract: Immunohistochemistry and melanin bleaching were used to assess the expression of antigens identified by anti-S-100 and anti-HMB-45 antibodies on melanomas and intramucosal and blue nevi from the oral mucosa of 18 patients. Both antibodies reacted with cells in all three types of lesions, but there were differences in the expression of these antigens between the round and spindle cells within the lesions. In melanomas composed of round cells, the intensity and distribution of staining with HMB-45 was greater than with S-100. The opposite was true in melanomas composed of spindle-shaped cells, and one spindle-cell melanoma was HMB-45-negative. The round cells of intramucosal nevi expressed S-100 more intensely and more frequently than HMB-45. The spindle-shaped cells of blue nevi strongly expressed both S-100 and HMB-45. Whereas intradermal nevi from the skin do not express HMB-45, intramucosal nevi consistently express this antigen in the lesion and overlying mucosa. Oral melanomas composed of round and spindle-shaped cells show differences in their expression of S-100 and HMB-45 antigens, making the use of both antibodies complementary in the diagnosis of undifferentiated tumors.

-Gorlach A, Herter P, Hentschel H, Frosch PJ, Acker H.
Effects of nIFN beta and rIFN gamma on growth and morphology of two human melanoma cell lines: comparison between two- and three-dimensional culture.

- Habeck JO.
Primary malignant melanoma of the gallbladder. Case report and literature review. Zentralbl Pathol 139(4-5):367-71, 1993.
Abstract: An autopsy case of a 57-year-old man with primary melanoma of the gallbladder is described. The tumour appeared as a polypoid mass, measuring 4 cm in diameter. Microscopic examination revealed mostly spindle cells with vesicular nuclei and large nucleoli. Many cells stained positively for S 100 protein and HMB 45 using immunohistochemistry and they contained dark brown pigment that stained as melanin pigment with Fontana-Masson. The tumour had metastasised and affected the stomach, the duodenum, the jejunum, the lung, the brain and a bronchopulmonary lymph node. Examination of the skin and both eyes showed no abnormality. Primary malignant melanomas of the gallbladder are very rare. Only 23 cases have been described. These cases are summarized and reviewed.

- Hara H, Walsh N, Yamada K, Jimbow K.
High plasma level of a eumelanin precursor, 6-hydroxy-5-methoxyindole- 2-carboxylic acid as a prognostic marker for malignant melanoma. J Invest Dermatol 102(4):501-5, 1994.

- Hara K.
Melanocytic lesions in lymph nodes associated with congenital naevus. Histopathology 23(5):445-51, 1993.
Abstract: Two cases of melanocytic lesions in lymph node associated with congenital naevus are presented. The first was a 30-year-old man with a nodular melanoma arising in a small congenital naevus. The second was a 2-year-old male infant with a giant congenital naevus. In both cases, naevus cell aggregates were observed in the capsule, trabeculae, perisinusoidal areas and lymphatic vessels surrounding the nodes. In the first case, clusters of large atypical melanocytes were present amongst naevus cell aggregates in the perisinusoidal areas as well as in the lymphoid parenchyma. Between the naevus cells and large atypical melanocytes, transitional forms were observed which supports the idea that the presence of large atypical melanocytes is indicative of benign naevus cells. In the second case, marginal sinuses were packed with clusters of large melanin -rich cells. Immunohistochemically, these cells were S-100 protein negative, but ultrastructural studies proved them to be melanocytes. They were considered indicative of spread of benign naevus cells via lymphatic vessels. Arrested migration of naevus cells during embryogenesis and benign spread of naevus cells are possible explanations for the histogenesis of naevus cell aggregates in lymph nodes associated with congenital naevus.

- Horikoshi T, Ito S, Wakamatsu K, Onodera H, Eguchi H.
Evaluation of melanin-related metabolites as markers of melanoma progression. Cancer 73(3):629-36, 1994.
Abstract: BACKGROUND Urinary excretion of 5-S-cysteinyldopa (5-S-CD) has been used as a biochemical marker of melanoma progression. Melanomas produce not only 5-S-CD but also 5,6-dihydroxyindole-2-carboxylic acid (5,6DHI2C) as major intermediates in melanin formation. 5,6DHI2C is then metabolized to the two O-methyl derivatives, 5H6MI2C and 6H5MI2C. The aim of this study was to determine which marker in serum and urine most sensitively reflected the progression of melanoma. METHODS. Serum and 24-hour urine samples were collected and assayed serially by high-performance liquid chromatography every 1 to 4 months in 28 patients with primary or recurrent melanomas, for up to 48 months. RESULTS. Serum concentration and urinary excretion of 5-S-CD and 6H5MI2C in patients with melanoma without metastases were close to those obtained from normal subjects. Metastases developed in 9 of the 28 patients. In seven of these nine patients, serum or urinary 5-S-CD values were elevated before or at the time of clinical detection of visceral metastases. However, serum 5-S-CD was elevated significantly earlier and reflected melanoma progression better than the physical examination and/or laboratory tests, such as scintigraphy and echography. Serum 6H5MI2C values exceeded the normal range shortly before death in three patients, and urinary 6H5MI2C did not increase at any stage in most patients, therefore these metabolites did not reflect progression of disease. CONCLUSIONS. Among the four markers, serum 5-S-CD appears to be the best biochemical marker for the detection of progression of melanotic melanoma, a value of more than 10 nmol/l suggesting the presence of metastasis.

- Hsiao GH, Hsiao CW.
Plaque-type blue nevus on the face: a variant of Ota's nevus? J Am Acad Dermatol 30(5 Pt 2):849-51, 1994.
Abstract: We describe a plaque-type blue nevus that had been present since birth on the right cheek of a 22-year-old man. It was characterized by several dark blue macules and papules with intervening areas of faint blue discoloration. Histologic examination showed a common blue nevus and a mongolian spot-like dermal melanocytosis in the dark blue and intervening faint blue pigmentary lesions, respectively.

- Ichikawa S, Nakajo N, Sakiyama H, Hirabayashi Y.
A mouse B16 melanoma mutant deficient in glycolipids. Proc Natl Acad Sci U-S-A. 91(7):2703-7, 1994.
Abstract: Mouse B16 melanoma cell line, GM-95 (formerly designated as MEC-4), deficient in sialyllactosylceramide was examined for

its primary defect. Glycolipids from the mutant cells were analyzed by high-performance TLC. No glycolipid was detected in GM-95 cells, even when total lipid from 10(7) cells was analyzed. In contrast, the content of ceramide, a precursor lipid molecule of glycolipids, was normal. Thus, the deficiency of glycolipids was attributed to the first glucosylation step of ceramide. The ceramide glucosyltransferase (EC 2.4.1.80) activity was not detected in GM-95 cells. There was no significant difference of sialyllactosylceramide synthase activity, however, between GM-95 and the parental cells. The deficiency of glycolipids in GM-95 cells was associated with changes of the cellular morphology and growth rate. The parental cells showed irregular shapes and tended to overlap each other. On the other hand, GM-95 cells exhibited an elongated fibroblastic morphology and parallel arrangement. The population-doubling times of GM-95 and the parental cells in serum-free medium were 28 hr and 19 hr, respectively.

- Kuwabara H, Uda H, Miyaguchi M, Nagai M, Saito K, Shibanushi T.
Pigmented squamous cell carcinoma of the alveolar ridge in the oral mucosa. Oral Surg Oral Med Oral Pathol 77(1):61-5, 1994.
Abstract: The clinical and morphologic features of a pigmented squamous cell carcinoma of the alveolar ridge in an 81-year-old Japanese woman are reported. The tumor was typical, well-differentiated squamous cell carcinoma but had many melanin -containing cells within it. Electron microscopy showed melanosomes in macrophages, melanocytes, and neoplastic squamous cells. Those in the neoplastic squamous cells seemed to have been excreted from the cytoplasmic processes of melanocytes.

-Marcus SG, Perry Lalley D, Mule JJ, Rosenberg SA, Yang JC.
The use of interleukin-6 to generate tumor-infiltrating lymphocytes with enhanced in vivo antitumor activity. J Immunother Emphasis Tumor Immunol. 15(2):105-12, 1994.

- Murphy MJ, Huang MY.
Q-switched ruby laser treatment of benign pigmented lesions in Chinese skin. Ann Acad Med Singapore 23(1):60-6, 1994.
Abstract: The Q-switched ruby laser has been demonstrated as an effective choice of treatment for a range of benign pigmented lesions. Its wavelength of 694 nm enables deep penetration of the skin allowing the treatment of both epidermal and dermal lesions. However, this wavelength is selectively absorbed by melanin thereby enabling efficient targeting of the lesion's melanocytes. By utilising a Q-switched pulsewidth of 25 nanoseconds, thermal conduction into surrounding tissues is minimised. Lesions such as nevus of Ota, chloasma, lentigines and cafe au lait have been successfully treated with energy densities ranging from 6 to 12 J/cm2. Four case histories are described in this report. The clinical evidence indicates that pigmented lesions in Chinese skin must be treated with energy densities higher than those used in Caucasian skin to minimise the incidence of hyper-pigmentation. Typically, lesions require a small number of treatments, usually within the range one to six, to effect complete removal. The technique is easy to apply, with no need for anaesthesia, in many cases.

-Pantazis P, Early JA, Mendoza JT, DeJesus AR, Giovanella BC.
Cytotoxic efficacy of 9-nitrocamptothecin in the treatment of human malignant melanoma cells in vitro. Cancer Res. 54(3):771-6, 1994.

- Phillips GL, Bundy BN, Okagaki T, Kucera PR, Stehman FB.
Malignant melanoma of the vulva treated by radical hemivulvectomy. A prospective study of the Gynecologic Oncology Group. Cancer 73(10):2626-32, 1994.
Abstract: BACKGROUND. Beginning in 1983, the Gynecologic Oncology Group (GOG) conducted a prospective clinicopathologic study of primary malignant melanoma of the vulva. The objectives of this study were to determine the relationship of histopathologic parameters and microstaging to the International Federation of Gynaecology and Obstetrics (FIGO) staging and prognosis. METHODS. All patients with primary untreated malignant melanoma of the vulva and no history of previous or subsequent other primary invasive malignancy were eligible for study entry. All patients were required to have modified radical hemivulvectomy as minimal therapy. Groin dissection was optional. Histopathologic specimens were reviewed for capillary space involvement, Clark's level, Breslow's depth of invasion, cell type, and melanin distribution. Patient characteristics were analyzed in their relationship to groin node status and recurrence-free interval. RESULTS. Between 1983 and 1990, 81 patients were entered in the study. Of these, 71 were evaluable. Thirty-four patients underwent radical hemivulvectomy, and 37 patients underwent radical vulvectomy. In addition, 56 patients underwent groin node dissection. The factors that were independently correlated with groin node status were: capillary lymphatic space involvement ($p = 0.0001$) and central primary tumor location (i.e., bilateral/clitoral/T3) ($P = 0.003$). The other factors that were significant--clinical tumor size, vulvar staging (FIGO), GOG performance status, and Breslow's depth of invasion--were not independent predictors of positive nodes. The factor with the highest significant correlation with recurrence-free interval was the 1992 staging system of the American Joint Committee on Cancer (AJCC) for malignant melanoma of the skin. Using multiple regression, AJCC stage was the only independent prognostic factor. In the absence of AJCC stage, Breslow's depth of invasion was the most prognostic. CONCLUSION. The biologic behavior of vulvar melanoma is similar to other nongenital cutaneous malignant melanoma.

- Qi Zirong, Liu Jinwei, Sun Ling.
Studies on the carrier of the wave spectrum signal during ESR diagnosis of malignant tumors. Bopuxue Zazhi 10:295-302, 1993.
Abstract: In the diagnosis of malignancy by the ESR technique, the authors obsd. that the signal of the ESR wave spectrum was stronger

for black hair than for white hair. The results show that human hair consists of 17 amino acids, the contents of which are identical in black and white hair, but the unique difference between black and white hair was shown to be melanin. The carrier of the ESR signal consists of melanin, protein, and lipid, and among them, melanin is the most important carrier of the ESR signal.

-Rodeck U, Bossler A, Graeven U, Fox FE, Nowell PC, Knabbe C, Kari C.
Transforming growth factor beta production and responsiveness in normal human melanocytes and melanoma cells. Cancer Res. 54(2):575-81, 1994.

- Rofstad EK, Steinsland E, Kaalhus O, Chang YB, Hovik B, Lyng H.
Magnetic resonance imaging of human melanoma xenografts in vivo: proton spin-lattice and spin-spin relaxation times versus fractional tumour water content and fraction of necrotic tumour tissue. Int J Radiat Biol 65(3):387-401, 1994.
Abstract: Proton nuclear magnetic resonance (1H-nmr) imaging is used routinely in clinical oncology to provide macroscopic anatomical information, whereas its potential to provide physiological information about tumours is not well explored. To evaluate the potential usefulness of 1H-nmr imaging in the prediction of tumour treatment resistance caused by unfavourable microenvironmental conditions, possible correlations between proton spin-lattice and spin-spin relaxation times (T1 and T2) and physiological parameters of the tumour microenvironment were investigated. Tumours from six human melanoma xenograft lines were included in the study. 1H-nmr imaging was performed at 1.5 T using spin-echo pulse sequences. T1- and T2-distributions were generated from the images. Fractional tumour water content and the fraction of necrotic tumour tissue were measured immediately after 1H-nmr imaging. Significant correlations across tumour lines were found for T1 and T2 versus fractional tumour water content ($p < 0.001$) as well as for T1 and T2 versus fraction of necrotic tumour tissue ($p < 0.05$). Tumours with high fractional water contents had high values of T1 and T2, probably caused by free water in the tumour interstitium. Fractional water content is correlated to interstitial fluid pressure in tumours, high interstitial fluid pressure being indicative of high vascular resistance. Tumours with high fractional water contents are thus expected to show regions with radiobiologically hypoxic cells as well as poor intravascular and interstitial transport of many therapeutic agents. T1 and T2 decreased with increasing fraction of necrotic tumour tissue, perhaps because complexed paramagnetic ions were released during development of necrosis. Viable tumour cells adjacent to necrotic regions are usually chronically hypoxic. Tumours with high fractions of necrotic tissue are thus expected to contain significant proportions of radiobiologically hypoxic cells. Consequently, quantitative 1H-nmr imaging has the potential to be developed as an efficient clinical tool in prediction of tumour treatment resistance caused by hypoxia and/or transport barriers for therapeutic agents. However, much work remains to be done before this potential can be adequately evaluated. One problem is that high fractional tumour water contents result in longer T1 and T2 whereas high fractions of necrotic tumour tissue result in shorter T1 and T2; i.e. the two parameters which are indicative of treatment resistance contribute in opposite directions. Another problem is that the correlations for T1 and T2 versus fraction of necrotic tumour tissue are not particularly strong.

-Ruck A, Jakobson E, Bjorkman S, Paulie S.
Adaptation of human bladder carcinoma cell lines to serum-free growth. Evidence for autocrine growth stimulation. Anticancer Res. 14(1A):55-60, 1994.

-Saiki I, Murata J, Yoneda J, Kobayashi H, Azuma I.
Influence of fibroblasts on the invasion and migration of highly or weakly metastatic B16 melanoma cells. Int J Cancer. 56(6):867-73, 1994.

-Stackpole CW, Groszek L, Kalbag SS.
Benign to malignant B16 melanoma progression induced in two stages in vitro by exposure to hypoxia. J Natl Cancer Inst. 86(5):361-7, 1994.

- Taylor CR, Flotte TJ, Gange RW, Anderson RR.
Treatment of nevus of Ota by Q-switched ruby laser. J Am Acad Dermatol 30(5 Pt 1):743-51, 1994.
Abstract: BACKGROUND: There are few reports on therapy for nevus of Ota. Moreover, traditional treatments are largely palliative or risk permanent pigmentary changes and/or scarring. OBJECTIVE: The efficacy of the Q-switched ruby laser (694 nm, 40 nsec) as a therapy for nevus of Ota was investigated. METHODS: Nine nevi or portions thereof were irradiated up to six times with 4.5 and/or 7.5 J/cm2 at a mean exposure interval of 3 weeks. Sequential skin biopsy specimens were processed for light microscopy, immunohistochemistry, and electron microscopy. RESULTS: Cosmetic improvement occurred at both doses in the irradiated parts of the six nevi available for follow-up. No appreciable difference was noted between single and multiple treatments. There was no gross scarring. Light microscopy revealed dose-related immediate injury with more melanophages and fewer dermal melanocytes after irradiation in comparison with control areas. Electron microscopic distinction between dermal melanocytes and melanin -laden macrophages was difficult. A monoclonal antibody to human melanosome-specific antigen type 1 (HMSA-1) was used to distinguish between the two cell populations. CONCLUSION: Our findings suggest that the Q-switched ruby laser is useful for treating nevus of Ota.

- Verrijk R, Smolders IJ, Huiskamp R, Gavin PR, Philipp KH, Begg AC.

Pharmacokinetics in melanoma-bearing mice of 5-dihydroxyboryl-6-propyl- 2-thiouracil (BPTU), a candidate compound for boron neutron capture therapy. Br J Cancer 69(4):641-7, 1994.
Abstract: Blood pharmacokinetics and tissue distribution of 5-dihydroxyboryl-6-propyl-2-thiouracil (BPTU), a boron carrier with postulated melanin -seeking properties for boron neutron capture therapy, were determined in C57/BL mice with subcutaneous pigmented or non-pigmented B16 melanomas. Borocaptate sodium (BSH) was used as a boron compound without melanin -seeking properties in a comparative biodistribution study in the same animal tumour models. Administration of single doses showed that BPTU was retained better in the pigmented B16 tumour than in the non-pigmented variant. BPTU was found in large concentrations in kidney and liver. Brain boron was approximately 10-fold lower than tumour boron. On a molar basis, BPTU demonstrated higher affinity for B16 tumours than BSH. Owing to solubility limits, tumour boron concentrations in this mouse study were too low for effective application of BNCT. However, the high tumour-to-blood and tumour-to-normal tissues ratios indicate that, with appropriate formulation, BPTU could be a promising candidate for clinical BNCT.

-Weber J, Salgaller M, Samid D, Johnson B, Herlyn M, Lassam N, Treisman J, Rosenberg SA.
Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2'-deoxycytidine. Cancer Res. 54(7):1766-71, 1994.
Abstract: MAGE-1 is a gene that encodes an antigen on a melanoma cell line that is recognized by cytolytic T-cells. We have used a reverse transcription-polymerase chain reaction assay to analyze expression of the MAGE-1 gene by cell lines from different types of tumors, melanomas from different stages of disease progression, normal diploid cell lines, and melanocyte and nevus tissue from which malignant melanomas are derived. MAGE-1 is expressed by melanoma tissue from all stages of disease, but not melanocytes, nevus tissue, or any normal diploid cell line tested. A fraction of tumor lines derived from various epithelial and neuroectodermal malignancies expressed MAGE-1 but not peripheral blood cells from patients with melanoma. 5-Aza-2'-deoxycytidine (DAC), a demethylating agent, was capable of inducing MAGE-1 expression by a MAGE-1-negative melanoma cell line 888-mel as well as by a number of other melanoma cell lines. At an optimum concentration of 1 microM DAC, MAGE-1 expression was detectable by 24 h, plateaued by 72 h, but remained high for two weeks after removal of DAC from treated 888-mel cells, consistent with induction by demethylation. With the exception of tumor-infiltrating leukocytes, no normal diploid cell line could be induced with DAC to upregulate MAGE-1 expression. DAC-treated 888-mel cells were lysed by a MAGE-1-specific major histocompatibility complex restricted cytolytic T-cell clone, whereas control untreated cells were not, suggesting that production of the antigen encoded by the MAGE-1 gene was induced by DAC and that it was presented in association with major histocompatibility complex class I molecules at the cell surface for T-cell recognition.

9. Eye

- Berges O, Cerezal L, Sterkers M, Mimoun G, Piekarski JD.
Collar-button choroidal melanoma. Anatomo-radiologic correlations. J Neuroradiol 21(1):50-5., 1994.
Abstract: Malignant melanomas of the choroid are the most frequent symptomatic eye tumours in adults. They often have a pathognomonic appearance, being collar-button or mushroom shaped due to rupture of Bruch's membrane by the tumoral mass. The ultrasonographic image of collar-button melanoma is well known: the head of the tumour is hyperechogenic and its base hypoechogenic. According to some authors, this is caused by difference in blood supply between the two parts. At MRI strongly pigmented melanomas emit a high-intensity signal on T1-weighted sequences and a low-intensity signal on T2-weighted sequences, but these characteristic features are inconstant. We present a case of collar-button melanoma explored by ultrasonography, colour Doppler Flow Imaging (CDFI) ultrasound and MRI, then enucleated. Flows and signals were different in front of, or behind the rupture of Bruch's membrane: ultrasounds showed a hyperechogenic image at the head and a hypoechogenic image at the base; on T2-weighted MRI sections intensity was greater in the head than in the base (head: 69 ms, base 180 ms) on CDFI, no flow was detectable in the head and very high flows were seen in the base of the tumour. Comparisons of these images with pathological findings, where there was no difference between head and base in melanin concentration and in cellular type (mixed or mainly epithelioid), led us to believe that the differences observed in images were essentially due to differences in blood supply between the two parts of the tumour constricted by the sides of the ruptured Bruch's membrane.

- Putting BJ, Van Best JA, Vrensen GF, Oosterhuis JA.
Blue-light-induced dysfunction of the blood-retinal barrier at the pigment epithelium in albino versus pigmented rabbits. Exp Eye Res 58(1):31-40, 1994.
Abstract: The purpose of this study was to determine the role of epithelial melanin in blue light phototoxicity of the retina. The first manifestation of the phototoxicity has been shown to be a breakdown of the blood-retinal barrier at the retinal pigment epithelium. The blood-retinal barrier function of six New Zealand albino rabbits was compared to that of four pigmented chinchilla rabbits after exposure to broad-band blue light (400-520 nm). Additionally, the spectral sensitivity of blood-retinal barrier dysfunction was determined by exposing 15 New Zealand albino rabbits to narrow-band blue light with peak intensity at lambda = 408 nm, 418 nm, 439 nm, 455 nm and 485 nm (bandwidth: 11.7-13.5 nm). The blood-retinal barrier function was evaluated with vitreous fluorophotometry. Ultrastructural changes and permeability of the retinal pigment epithelium for horseradish peroxidase were evaluated in the albino rabbits with electron

microscopy. Exposure to broad-band blue light up to 832 J cm-2 demonstrated the blood-retinal barrier of albino and pigmented rabbits to be equally sensitive. Electron microscopy of albino rabbits exposed to above-threshold energy demonstrated an increase of inclusion bodies in the retinal pigment epithelium and vacuolation of the cytoplasm. Transcellular passage of intra-arterially administered horseradish peroxidase through the pigment epithelium into the subretinal space was seen. The narrow-band exposures demonstrated that light of 439 nm was more effective than the light of other wavelengths in inducing barrier dysfunction in albino rabbits. This implies that chromophores absorbing at 439 +/- 6 nm were responsible for the phototoxicity in albino rabbits. The results indicate that melanin does not have a damaging nor a protective role in phototoxicity since (1) the presence of melanin is not essential for blue-light-induced photochemical damage to the blood-retinal barrier at the retinal pigment epithelium, and (2) protection from this sort of damage is not greater in melanin containing epithelia than in non- melanin containing epithelia.

- Salazar-Bookaman MM, Wainer I, Patil PN.
Relevance of drug-melanin interactions to ocular pharmacology and toxicology. J Ocul Pharmacol 10(1):217-39, 1994.
Abstract: In melanocytes, the biosynthesis of L-dopa derived indole polymer, melanin , is accelerated by tyrosinase and related enzymes. The brown to black pigment is characterized by a stable free-radical property. In humans, a pigment dependent slow onset of ocular actions of ephedrine, atropine, cocaine, pilocarpine and related medications was observed. Extensive accumulation of drugs by melanin appears to be the most important factor governing the long term therapeutic/toxicological activities. Drugs crossing placental circulation are localized in the mouse fetal eye. Thus, drugs exhibit a high binding capacity for melanin containing tissues. Studies on synthetic melanin and melanin granules also indicated a high binding capacity of many therapeutic classes of drugs, including psychotropics. In addition to the liposoluble property of the molecule, there is a definite relationship between chemical structure and the affinity of drugs for melanin . For example, the affinity of chlorpromazine for melanin is higher than that of chlorprothixene. NMR studies, with soluble melanins indicate that there is a steric preference among ephedrine enantiomers. A high binding capacity indicates that more than two molecules of (-)-ephedrine may complex with one indole unit of melanin . Ocular drug development calls for the study of qualitative and quantitative aspects of drug- melanin interaction.

10. Other

- Hegedus ZL, Nayak U.
Renal excretion of plasma soluble melanins by healthy human adults. Arch Int Physiol Biochim Biophys 101(6):417-23, 1993.
Abstract: The soluble melanins of blood plasma form in vivo and in vitro from dopa, catecholamines, catechol, hydroquinone, homogentisic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, p-aminophenol, p-phenylenediamine and other structurally related end(ex)ogenous compounds by oxidative polymerization. The mean quantity of natural melanins in normal plasma is 1.61 +/- 0.10 (standard deviation) mg/ml, (n = 20) and in uraemic plasma 2.72 +/- 0.38 mg/ml, (n = 16). The plasma melanins (approximately 3%), are associated with proteins (approximately 85%), mucoproteins (approximately 0.25%), lipids (approximately 0.4%), as soluble lipofuscins, and probably are associated with proteins without lipids as soluble melanoproteins. Fluorescence, UV-VIS and IR spectroscopies and the melanin isolation method show the presence of soluble melanins in the urine of healthy people. Soluble melanins can also be formed in vitro in the urine by oxidative polymerization of the precursors. In most of the urine samples we studied, melanins were present in larger amounts than the urinary proteins, indicating that the kidneys can selectively excrete the melanin components of the lipofuscins, and that the solubility of melanins does not depend upon combination with proteins. The quantities of purified melanins precipitated with 6 N HCl at 110 degrees C during 72 h from urine samples collected during 24 h periods ranged from 0.1460 g to 3.7627 g (mean 1.1303 +/- 1.1739 g, n = 8) and the plasma clearance rates ranged from 0.06 ml/min to 1.56 ml/min (mean 0.48 +/- 0.48 ml/min, n = 8). From the individual 24 h urine samples we obtained from 9 to 216 mg/dl of precipitated melanins while the individual plasma samples contained from 145 to 175 mg/dl.

ANNOUNCEMENTS & RELATED ACTIVITIES

PIGMENT CELL RESEARCH

Publisher's Note:

1995: A year of change for *Pigment Cell Research* as Joseph T. Bagnara's term as Editor expires and Professor Takuji Takeuchi assumes editorship.

Pigment Cell Research, now in its seventh year, is about to undergo a planned change of editorship. It is a change that Founding Editor Joseph T. Bagnara sees as a natural evolution in the history of the journal.

Joseph T. Bagnara's first and foremost reason for starting *Pigment Cell Research* was to fill the need for a first-rate outlet for original papers in the field of pigment cell biology. In 1986 he took the idea to the late Alan R. Liss and in 1987 the first issue was published. The purchase in 1988 of Alan R. Liss, Inc., by John Wiley and Sons, Inc., momentarily brought some uncertainty to the journal's future, but Munksgaard International Publishers saw an opportunity in the publication. With Munksgaard's purchase of the journal, Joseph T. Bagnara entered into the new cooperation with an enthusiastic and open mind. We at Munksgaard quickly learned to appreciate his deep involvement with and concern for the journal.

In 1991, the Agreement between the International Federation of Pigment Cell Societies and Munksgaard International Publishers was formalized and the journal has since been sponsored by the Federation, which consists of the European, the Japanese, and the Pan American Societies for Pigment Cell Research.

Joseph T. Bagnara is now completing seven years as Editor-in-Chief. By building the strengths and traditions of the journal, he has played a major part in placing *Pigment Cell Research* in its present esteemed position. it is with profound respect and admiration that I express the publisher's gratitude for Joe's excellent and invaluable work as Founding Editor of Pigment Cell Research.

The editorship of the journal will pass to Professor Takuji Takeuchi, a renowned scientist within the field of pigment cell research who has a strong background in molecular biology, developmental biology, and genetics. We are confident that the journal will continue to improve under his leadership and it is with great pleasure that we welcome Professor Takuji Takeuchi as the new Editor-in-Chief of *Pigment Cell Research*.

Hanne Freno, Editor
Munksgaard International Publishers
Journals Division

With immediate effect, contributors are kindly requested to submit their papers to

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Information about the XVIth IPCC

1.Satellite Conferences: No satellite conference will be supported by the local organizing committee that are held within the time frame of the XVIth International Pigment Cell Conference, Tuesday, October 29, 1996; 6:00 p.m. to Sunday, November 3, 1996; 8:00 a.m. There are a wide number of venues possible to hold small or large satellite conferences either before or after the main pigment cell meeting. Our Memorial/UCI Educational Foundations will be happy to work with you in planning, for a small fee, and we request that we be notified of the intent of any satellite conference no later than June 1, 1995. If we are notified later than this dat, accommodations and planning availability cannot be guaranteed.

2.Competitive Stipend for Travel Support: The Organizing Committee will provide funds in a competitive manner for graduate students, post doctoral fellows and those within five years of formal academic appointment. The number of stipends will depend on the availability of funds and further information will become available during the second and subsequent informational mailings.

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NEWS FROM THE ESPCR

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

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INTERNATIONAL FEDERATION OF PIGMENT CELL SOCIETIES

MESSAGE FROM THE PRESIDENT

Dear Colleagues,

At the last International Pigment Cell Conference in London I was appointed as President of the International Federation of Pigment Cell Societies (IFPCS) and I thought it was now the time to share with you my feelings as to how the Federation should move in the coming years. Before doing that, however, I would like to express my appreciation to my predecessor, Yutaka Mishima, the General Secretary, Jim Nordlund and the other Council members for all the work done in the first three years of the fledgling IFPCS. As freely admitted by Professor Mishima, those years still beared with them the marks of the long and troubled gestation, which preceded the establishment of the Federation in Kobe. Yet, the commitment and collaborative spirit of all the officers and Council members have gradually smoothed away the difficulties and have eventually steered the Federation to the calm waters where it presently stands.

Time is therefore ripe for bringing the Federation to a new developmental phase, where each of the regional societies concurs to the furtherance of common objectives in an authentic federalistic spirit. Paradoxically, a major difficulty to this aim may come from the growth of strong regional societies which has somewhat favoured a compartmentalisation of scientific interactions based on merely geographic criteria. Although an opportunity for aggregation is offered by the International Pigment Cell Conferences, in recent years the pace of pigment cell research has increased bewilderingly to a point that these events can no more cover all the exigencies for exchange of information and cooperation. It is therefore clear that my primary effort will be directed to confer the Federation its full physiognomy of a super partes society, providing an organisational support to promote research on a global level without infringing the autonomous functioning of the regional societies.

One approach to this goal is to establish a number of International Expert Committees, that should serve as authoritative reference bodies to coordinate research, report on progress, settle controversial issues, and form connecting networks on those areas of pigment cell research deserving of special attention. This idea was submitted and unanimously approved during the last Council meeting of the Federation in Philadelphia. As a result, three research areas have been identified, namely **Albinism, Vitiligo and Comparative Biology of Pigmentation**. Richard King, Jim Nordlund and Sally Frost have been appointed as coordinators of the relevant Expert Committees. These should be optimally composed by up to 6 members, 2 from each regional society, but are open to suggestions and inputs from all those who are interested in the related fields. Work is currently underway to establish similar Expert Committees on other topical areas, such as Age Pigments, Photobiology of Pigmentation and Biophysics of Melanins.

Concurrent with these projects, a Committee has also been established to collect data on resources available to the pigment cell community. That Committee consists of Vince Hearing (chair), Patrick Riley and Takuji Takeuchi, and is aimed at establishing an interactive data base, to be known as the **"International Data Bank of Pigment**

Cell Resources". Listed in that data base will be all reagents, probes and other materials available and specifically used in research targeted towards pigment cells, including those useful in chemical, biochemical, immunological and molecular biological studies. Information will be forthcoming to members as to how the data will be collected and collated, and how it can eventually be accessed by any IFPCS member.

Most members of the Council have also emphasised the need to have a Committee for standardising nomenclature related to pigment genes and the pigment pathway, as the knowledge explosion continues. We all agreed that the IFPCS should be at the forefront of leading the discussions and helping set the criteria for appropriate nomenclature, so steps are being taken along this line. Of course, any comment or suggestion is warmly welcome and will be taken in due consideration by the Council.

Finally, efforts are being made to further support the journal **Pigment Cell Research**. As most of you are probably aware, starting from January 1995 Professor Takeuchi will replace Professor Bagnara as Editor-in-Chief. After many years of dedicated work, we wish to sincerely thank and congratulate the past Editor Joe Bagnara on the excellent job made in collaboration with the Publishers Alan Liss, Inc and Munksgaard. Pigment Cell Research has thus far served as a useful forum for publication of papers in all areas relevant to the title of the journal, and we hope that this positive trend will continue under the new Editorship. We wish Professor Takeuchi a bright success in making Pigment Cell Research the vehicle of choice for the publication of articles at the cutting edge of modern investigation.

In closing, I believe that all these initiatives, if thoroughly pursued, will have a tremendous impact on pigment cell research and will be highly beneficial for expanding the breadth of the Federation, increasing its visibility and enrolling new members to the sister societies. Our team is very active and has all the necessary requisites of experience, energy and judgement to confer the Federation an image of scientific excellence all over the world. But, of course, little can be done without the inputs and active cooperation of all members.

With my best wishes.

Giuseppe Prota

SURVEY OF CURRENT PIGMENT CELL RESEARCH IN EUROPE
1993-1994

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