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

Discussion

Melanin-related metabolites as melanoma markers

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The incidence of malignant melanoma is increasing worldwide and Japan is no exception. Three hundreds Japanese died of melanoma in 1985 and the number had doubled in the past 20 years (1). The diagnosis and treatment at the early stage are important for patients with malignant melanoma. Furthermore, the progression of melanoma is critical for patients at the advanced stages. Thus, tumour markers that sensitively detect metastasis have long been awaited.

Two types of melanin pigments, the black eumelanin and the reddish-brown pheomelanin, are produced not only in melanocytes but also in melanoma cells (2). They are formed from 5,6-dihydroxyindoles or cysteinyldopas (CD) through the tyrosinase oxidation of tyrosine in the absence or presence of cyteine, respectively. Major portions of these melanin precursors may be oxidized to yield the melanin pigments. However, minor portions may be leaked into the blood stream, partly O-methylated in the liver, and excreted into the urine. Thus, it would be possible to estimate the progression of melanoma by measuring the concentrations of these melanin-related metabolites in blood or urine (3). In fact, the urinary excretion of the major isomer of cysteinyldopas, 5-S-CD, has been used as a biochemical marker of melanoma in some countries, especially in Sweden (4). On the other hand, clinical significance of the indolic metabolites such as 5(6)-hydroxy-6(5)-methoxyindole-2-carboxylic acid (5H6MI2C and 6H5MI2C) has also been suggested (5).

Several years ago, we started comprehensive studies to evaluate the clinical significance of these melanin-related metabolites as markers of melanoma progression (for our latest publication, see ref. 6). In this "discussion", the recent progress in this field and my view regarding the clinical significance of 5-S-CD and 6H5MI2C in melanoma patients will be summarized briefly.

Brief history of 5-S-CD as a marker of melanoma : the high level of urinary excretion of 5-S-CD in patients with melanoma was first reported in 1973 and since then many groups have studied its clinical significance. The Swedish group has published a paper summarizing the results on 571 melanoma patients (4). Of 161 patients with metastases 60% showed values exceeding the pathological level of 400 μg/day (1.3 μmol/day) while less than 2% of 410 patients without metastases exceeded this level.

In recent years 5-S-CD in plasma (or serum) has drawn more attention. Significantly higher levels of plasma 5-S-CD were found not only in patients with extraregional metastases (stage IV) but also in those with regional lymph node metastases (stage III) (7).

5-S-CD genesis in melanocytes and other normal cells : cysteinyldopas are precursors of pheomelanin. However, the presence of cysteinyldopas does not necessarily indicate pheomelanogenesis, as will be discussed below.

During the sunny season urinary excretion of 5-S-CD is significantly increased. Thus, the mean value of excretion in the Swedish people in summer was threefold greater than that in winter (8). This has been considered as a major limitation of 5-S-CD in following up melanoma patients. We have also observed a seasonal variation in the excretion of 5-S-CD in Japanese (9). It should be stressed, however, that the degree of variation in Japanese was not so pronounced as in people living in Sweden.

The finding that albino mice excreted as much 5-S-CD as black mice led us to speculate the extramelanocyte origin of cysteinyldopa genesis (10). We have found substantial amounts of dopa and 5-S-CD in the hydrolysates of non-melanogenic tissues such as liver and kidney from mice and have thus indicated that 5-S-CD in normal subjects might be derived mostly from the protein-bound 5-S-CD that is produced by non-specific oxidation taking place on the tyrosine residue in proteins (10). In accordance with this, plasma 5-S-CD concentrations in both tyrosinase-positive and negative albino patients were found to be almost identical to the control values (11). It has also been shown that urinary excretion of 5-S-CD is independent of skin color (12). Furthermore, no correlation was found between the basal 5-S-CD excretion and skin type or number of melanocytes, and subjets with skin type II had a significantly higher 5-S-CD excretion than those with skin type III-IV after UV-B exposure (13). These results led the authors to suggest that the increase in 5-S-CD excretion during UV irradiation is due to UV damage of melanocytes rather than to the stimulation of melanogenesis (13). It now appears that Japanese are less vulnerable to sun exposure than fair-skinned Caucasian and have thus the serum (plasma) 5-S-CD levels less variable throughout a year. Our ongoing research is in support of this view.

5-S-CD genesis in melanoma cells : next problem to be considered is the production of high levels of 5-S-CD in eumelanic melanoma. B16 melanomas contain almost pure eumelanin, yet they produce as much 5-S-CD as the eumelanin-related metabolite 6H5MI2C (14). Two explanations seem to be possible. One hypothesis is that the tyrosinase leaked from melanosomes catalyzes dopaquinone production in the cytoplasm leading exclusively to cysteinyldopa genesis. In fact, the soluble fractions from B16 and

Harding-Passey melanomas contained greater concentrations of 5-S-CD than the melanosome fractions (15). The other hypothesis is that the defect of membrane in abnormal and incomplete (aberrant) melanosomes found in melanomas facilitates leakage of dopaquinone into the cytoplasm (16). Whatever the mechanism of cysteinyldopa genesis, these results suggested that although 5-S-CD has no value as an indicator of melanogenesis in normal melanocytes, it is a good indicator of the tyorsinase activity in melanoma cells and thus of the progression of melanoma (Table).

6H5MI2C as a marker of melanogenesis : 5,6-dihydroxyindole (5,6DHI) and its carboxy derivative (5,6DHI2C) are intermediates of eumelanogenesis. However, these dihydroxyindoles cannot be used as biochemical markers, because they are extremely labile to oxidation and most of the indoles are O-methylated either in melanoma or liver (5). Thus, the O-methyl derivatives of 5,6DHI and 5,6DHI2C may instead serve as markers reflecting the degree of eumelanogenesis or the progression of melanoma.

In contrast to 5-S-CD, the eumelanin-related metabolite 6H5MI2C (or 5H6MI2C) has been shown to reflect well the skin type and hair color (17). There have been no reports studying the seasonal variation in the levels of these indoles. However, it has been shown that after PUVA treatment the urinary excretion of both 5-S-CD and 6H5MI2C elevated several-fold and paralleled with each other (18). These results suggest that 6H5MI2C can be used as a marker of melanogenesis.

Comparison of 5-S-CD and 6H5MI2C as melanoma markers : we estimated normal values from 33 Japanese. The mean values of urinary excretion of 5-S-CD and 6H5MI2C were found to be both ca 0.4 μmol/day and those of serum concentrations ca 4 nmol/L (6). Levels of these markers in urine were much more variable than those in serum. Considering the wide variation of these values, we have adopted 1.5 μmol/day and 10 nmol/L as the upper limits of normal values for both markers. The urinary excretion of both 5-S-CD and 6H5MIC2 decreased in elderly subjects, while their serum concentration showed no age-dependent differences. This might be ascribed to the decrease of renal clearance associated with aging.

In collaboration with Dr. T. Horikoshi, Dept of Dermatology, Sapporo Medical College, we have been carrying out since 1989 a comparative study to determine which of the 4 markers (Table) reflects most sensitively the progression of melanoma (19). Among more than 30 patients with primary or metastatic melanoma, eigth had advanced to the stage IV and eventually died. In those 8 patients serum 5-S-CD values had been elevated to the pathological level before or when the metastases were detected clinically. In 7 of the 8 cases the elevation of 5-S-CD was more pronounced in serum than in urine. 5-S-CD and 6H5MIC2 levels in the remaining patients without metastasis were within normal ranges. Serum 6H5MI2C exceeded the normal range only at the end stage in 2 patients. Urinary excretion of 6H5MI2C did not reflect the progression of disease.

HPLC measurements of 5-S-CD and 6H5MIC2 : the concentration of 5-S-CD in urine or serum has usually been determined by HPLC with electrochemical detection. With the original method using alumina to extract 5-S-CD from serum (20), it was not possible to remove3,4-dihydroxypenylacetic acid (DOPAC) which is eluted out much slower (52 min) than 5-S-CD (12 min). We have recently developed a new method to overcome this

difficulty by washing alumna with an acidic buffer prior to the elution of 5-S-CD with perchloric acid (to be published). It has now become possible to analyze more than 20 serum or urine samples within 24 hrs using HPLC equipped with an automated sampler. This can be compared to the on-line automated HPLC procedure for the determination of 5-S-CD in urine (21).

Conclusion and perspective

It now became apparent that 5-S-CD in serum reflects the progression of melanoma more sensitively than in urine. It is possible to expect that serum 5-S-CD gives more direct informations on melanogenic activity of melanoma. Urinary 5-S-CD is, on the contrary, more influenced by metabolic activities, such as oxidation, O-methylation, and cojugation, that may be taking place in tissues other than melanoma. Furthermore, it should be stressed that serum sample is much easier to collect than 24-hr urine sample. This is particularly important for following up outpatients with suspected melanoma metastases.

We have just started a comprehensive and collaborative project involving dermatology departments of more than 60 universities and cancer institutes in Japan to evaluate in melanoma patients the clinical significance of serum 5-S-CD as a marker of 1) the progression of disease (detection of metastasis); 2) the efficacy of therapy; 3) the prognosis.

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Table : Comparison of 5-S-CD and 6H5MI2C in serum and urine

	Serum		Urine	
	5-S-CD	6H5MI2C	5-S-CD	6H5MI2C
Clinical and biological significance				
Melanoma progression[a]	Better	Poor	Good	Poorer
Melanogenesis in skin	Poor	Not known	Poor	Good
Measurement				
HPLC pretreatment	Moderate	Difficult	Moderate	Easy[b]
HPLC reproducibility	Moderate	Low	Moderate	High
HPLC time (min)[c]	40	40	40	40
Sample collection	Easy		Troublesome	

[a] : in Japanese
[b] : no pretreatment required
[c] : for repeated injections

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CURRENT LITERATURE

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

- Akiu S, Suzuki Y, Asahara T, Fujinuma Y, Fukuda M.
Inhibitory effect of arbutin on melanogenesis. Biochemical study using cultured B16 melanoma cells. Nippon Hifuka Gakkai Zasshi 101:609-613, 1991.
Abstract : Melanin formation and activity of tyrosinase were decreased by addn. of arbutin (5 .times. 10-5 M) to the incubation medium of murine B16 melanoma cells without influence on the cell growth compared to those of arbutin-untreated cells. The B16 melanoma cell suspension did not hydrolyze arbutin. Enzyme activity in the crude prepns. from the B16 melanoma cells was dose-dependently inhibited by arbutin. Apparently, arbutin inhibits melanogenesis by affecting not only synthesis but also the activity of tyrosinase, in which hydroquinone is not involved.

- Aliev GA, Ivanov AV, Rachkovskii ML.
ESR study of copper(II) complexes with eumelanin and pheomelanin. Dokl Akad Nauk SSSR 318:606-609, .1991.
Abstract : The ESR spectra are reported of Cu(II) complexes with eumelanin and pheomelanin. In eumelin the g-factor is 2.058. The Cu atom in eumalin is in a distorted square-planar coordination with 2 ring N atoms and 2 keto O atoms in trans positions. An phenomelanin, the same coordination is obsd. and is so distorted that it is tetrahedral.

- Bilinska B, Simonovic B, Wilczok T, Vucelic D.
Melanin structure from banana peel by infrared spectroscopy. Biol Nauki (Moscow) 30-38, 1991.
Abstract : Melanin-like pigments were isolated from blackened banana peel. An anal. of the structure and phys. and chem. properties of these pigments as well as of synthetic dihydroxyphenylethylamine-melanin (DA-melanin) and humin formed during sucrose oxidn. was done by IR spectroscopy. The melanins of banana peel showed high thermal stability and structural similarity to synthetic DA-melanin. It was suggested that blackened banana peel also contains other melanin-like pigments (humins).

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Some remarks on the biochemical specificity of malignant melanoma. Wiss. Beitr.- MartinLuther-Univ. Halle-Wittenberg, (41, Malig. Melanom), 89-92, 1989.
Abstract : A review with 3 refs. which briefly discusses urinary melanogens in malignant melanoma.

- Garvey W, Fathi A, Bigelow F, Jimenez C, Carpenter B.
A new method for melanin at acid pH. J Histotechnol 13:279-282, 1990.
Abstract : A simple, reliable, consistent, and economical nonbuffered acid method for melanin is presented. After a short presensitization in Ag, the sections are treated in gum mastic and then immersed in a phys. developer consisting of hydroquinone, gum mastic, and a very low concn. of AgNO3. The sections are placed in thiosulfate and counterstained with Nuclear Fast Red. The entire procedure takes under 40 min to perform.

- Hegedus ZL, Nayak U.
Para-aminophenol and structurally related compounds as intermediates in lipofuscin formation and in renal and other tissue toxicities. Arch Int Physiol Biochim Biophys 99:99-105, 1991.
Abstract : P-aminophenol is considered a minor nephrotoxic metabolite of phenacetin and acetaminophen (paracetamol) in man. Our experiments show that p-aminophenol readily undergoes oxidative polymerization during incubation in human blood or plasma, to form melanin, as a component of soluble lipofuscin. Haemolysis accompanies this process in whole blood. Unmetabolized phenacetin and acetaminophen do not form soluble lipofuscins. Long-term excessive use of phenacetin or acetaminophen has been associated with chronic renal disease, haemolytic anaemia, and increased solid lipofuscin deposition in tissues. Excessive use of phenacetin has also been associated with cancer of renal pelvis and bladder. It appears to us that p-aminophenol and other o- and p-aminophenol metabolites of these drugs are intermediates not only in the etiology of chronic renal disease, but in the other developments as well. P-aminophenol and other ex(end)ogenous aminohydroxyphenyl, aminopolyhydroxyphenyl, polyhydroxyphenyl and polyaminophenyl compounds with these groups in ortho and para positions (such as 3-hydroxyanthranilic acid, 6-aminodopamine, dopamine, p-phenylenediamine, etc.) can undergo autoxidations and metal-catalyzed and enzymatic oxidations in man to produce toxic (semi)quinones(imines), (semi)quinonediimines and reactive oxygen species. After depletion of antioxidants these very reactive (semi)quinones(imines) and (semi)quinonediimine intermediates, many of which are precursors of plasma soluble lipofuscins and melanoproteins, react with essential proteins, DNA, other macromolecules and can cause or contribute to renal and other tissue toxicity, haemolytic anaemia, neoplasia, and granular lipofuscin formation. The reactive oxygen species can also deplete antioxidants, damage essential proteins, DNA, and other macromolecules, and thereby injure cells and extracellular matrix.

- Jacobson ES, Emery HS.
Temperature regulation of the cryptococcal phenoloxidase. J Med Vet Mycol 29:121-124, 1991.
Abstract : Melanin formation at 37 degrees C has been proposed as a virulence factor in Cryptococcus neoformans. However, whereas catecholamine uptake is maintained at this temperature, phenoloxidase, which catalyses the oxidation of catecholamine to melanin, is severely decreased in most wild type strains cultivated at 37 degrees C.

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

- Nikolaev ON, Aver'yanov AA.
Role of the superoxide radical in the mechanism of fungicidal action of phthalide and probenazole. Fiziol Rast (Moscow) 38:512-520, 1991.
Abstract : Phthalide at 5 .mu.g/mL Pyricularia oryzae inoculum or probenazole drench at 40 mg/kg soil 2 days before excision of rice leaf fragments, prevented blast development in the fragments and stimulated the release of active oxygen species in fungitoxic concn. Phthalide inhibited the antioxidative system (melanin and the enzymes superoxide dismutase and catalase) of the pathogen, which probably made it more sensitive both to toxic excretions from leaves and high light intensity. The capacity of chems. for stimulating generation of active oxygen species by the host plant and/or increasing the pathogen sensitivity to these plant metabolites may be used for developing new fungicides.

- Petelina GG, Dontsov AE, Ostrovskii MA, Lapikova VP, Aver'yanov AA, Dzhavakhiya VG.
The ESR study of pigments of phytopathogenic fungus Pyricularia oryzae Cav. Biol Nauki (Moscow) 76-80, 1991.

Abstract : The concn. of paramagnetic centers in mycelia, spores, and pigment isolated from different strains of the title phytopathogenic fungus has been studied. A mutant with defective pigmentation and having rose-colored mycelium has an ESR signal similar to that of a melanin-forming strain. The relation of fungal melanin with pathogenicity is discussed.

- Ren J, Ren R, Shen D.
Synthesis of hydroquinone carboxylates for depigmentation of skin. Zhongguo Yiyao Gongye Zazhi 22:150-151, 1991.
Abstract : p-ROC6H4OR1 (I; R, R1 = H, Me, Et, R = R1 .noteq. H) were prepd. by esterification of hydroquinone with acid chlorides in the presence of 4-(dimethylamino)pyridine and Et3N. I (R = H, R1 = Et) showed 80% efficiency in removing melanin, vs. 54.6% with hydroquinone.

- Steiner K, Buhring KU, Merck E.
The melanin binding of bisoprolol and its toxicological relevance. Lens Eye Toxic Res 7:319-333, 1990.
Abstract : Unexpectedly high accumulations of bisoprolol were detected in iris and ciliary body and in retina+choroid of beagles after 4 weeks of conjunctival and oral administration. This phenomenon gave reason to assume that these high concentrations in the pigmented structures of the eye might be related to melanin binding. According to literature a series of drugs, e.g. chloroquine, rifampicine, chlorpromazine, benzodiazepines, and also beta-adrenoceptor antagonists exhibit melanin-binding properties. By means of autoradiography it could be demonstrated in pigmented mice that after iv and po administration 14C-labelled bisoprolol was selectively bound to the melanin-containing parts of the eye, irrespective of the mode of administration. Since the melanin-bound radioactivity could be extracted from the eye of mice and was eliminated with a t1/2 of approx. 7 days, the melanin binding of bisoprolol is considered to be reversible. Other beta-blocking agents like timolol and befunolol used already for a long time in the therapy of glaucoma have been reported to bind specifically to the melanin of the eye, and show comparable long half-lives, similar to bisoprolol. Usually, autoradiographic studies on drug distribution are performed with albino animals. This leads to a lack of information on melanin binding and may result in misinterpretation concerning the retention of substances, especially in pigmented compartments of the eye. Therefore, in autoradiographic studies of new investigational drugs during preclinical development, one should use both pigmented and albino animals.

2. Biology of pigment cells and pigmentary disorders

- Balentien E, Mufson BE, Shattuck RL, Derynck R, Richmond A.
Effects of MGSA/GRO alpha on melanocyte transformation. Oncogene 6:1115-1124, 1991.
Abstract : In the work described here we demonstrate that the clonal cell line Mel-a-6, produced by transfection of mouse Melan-a cells with human MGSA, had an increased ability to form large colonies in soft agar and increased ability to form tumors when injected into nude mice as compared to cells transfected with the neomycin resistance gene alone. This effect appeared to be dependent on the levels of MGSA produced since another transfected clone, Mel-a-l, produced only a low level of MGSA transgene mRNA, formed only minimal large colonies in soft agar and had a tumorigenic rate equal to that of neomycin resistant controls. The histology of the Mel-a-6 tumors is compatible with features normally exhibited by melanoma tumors. The cells do not stain for melanin, and they are positive for the neural crest marker protein S-100 as well as the HMB 45 melanoma specific antigen. Immunohistochemical studies revealed expression of the human MGSA in tumor cells from tissues, excised from animals injected with cells from clone Mel-a-6. Whereas DNA ploidy analysis suggests that in vitro the Mel-a parent cell line, control Mel-a-neo, Mel-a-1 and Mel-a-6 cells show no evidence of aneuploidy, the nuclei isolated from the tumors from animals injected with Mel-a-6 do exhibit aneuploidy. These data suggest that over-expression of MGSA in immortalized melanocytes contributes to transformation.

- Brooks G, Birch M, Hart IR.
Effects of biologically active tumor-promoting and non-promoting phorbol esters on in vitro growth of melanocytic cells. Pigm Cell Res 3:98-100, 1990.
Abstract : Sapintoxin A (SAP A), a naturally occurring biol. active but non-promoting phorbol ester, acts as an effective in vitro mitogen for freshly derived human melanocytes. Seven days after addn. of 50 nM SAP A, there was a four to fivefold increase in melanocyte no. over that obsd. in untreated control cultures comparable to that achieved with a 50 nM concn. of 12-O-tetradecanoylphorbol 13-acetate (TPA). The fluorescent stage 2 promoter sapintoxin D also supported the growth of these cells, with a 50 nM dose producing an increase in cell no. comparable to that obsd. with 200 nM TPA. Similar results were obtained with an established, but non-tumorigenic, line of murine melanocytes. The same compds. exerted a potent anti-proliferative effect against transformed melanocyte lines of murine and human origin assocd. with morphol. alterations and an increase in melanin prodn. consistent with induced cytodifferentiation.

- de Vries H, Penninks AH, Snoeij NJ, Seinen W.
Comparative toxicity of organotin compounds to rainbow trout (Oncorhynchus mykiss) yolk sac fry. Sci Total Environ 103:229-243, 1991.
Abstract : The comparative toxicity of various organotin compounds was investigated in early life stages of the rainbow trout. Beginning with yolk sac fry, trout were continuously exposed for 110 days to tributyl- (TBTC), triphenyl- (TPhTC) or tricyclohexyltin chloride (TCHTC) at concentrations of 0.12-15 nM, to trimethyltin chloride (TMTC) at concentrations of 3-75 nM or to dibutyl- (DBTC) or diphenyltin chloride (DPhTC) at 160-4000 nM. The diorganotin compounds DBTC and DPhTC were about three orders of magnitude less toxic than the triorganotin homologs TBTC and TPhTC. Both for DBTC and DPhTC, a no observable effect concentration (NOEC) of 160 nM was established, corresponding to 40 and 60 ppb, respectively. Of the triorganotin compounds, TCHTC appeared to be the most toxic, inducing 100% mortality within 1 week at a concentration of 3 nM. Only a few trout survived exposure to 0.6 nM TCHTC for 110 days. TBTC and TPhTC caused acute mortality at a concentration of 15 nM. For both TBTC and TPhTC a NOEC of 0.12 nM was established, corresponding to water concentrations of 40 and 50 ppt, respectively. Histopathological examination revealed depletion of glycogen in liver cells of both di- and triorganotin exposed fish, except in the case of TMTC. No signs of toxicity were observed in fish exposed to up to 75 nM TMTC, the highest concentration tested. Atrophy of the thymus, the most prominent sign of toxicity of di- and tributyltin compounds in mammalian species, was not observed in early life stages of rainbow trout. Tail melanization was observed in the groups exposed to 3 nM TPhTC, 3 nM TBTC, 800 nM DBTC and 800 nM DPhTC. At the end of the exposure period, resistance to infection was examined by an intraperitoneal challenge with Aeromonas hydrophila, a secondary pathogenic bacterium to fish. Resistance of bacterial challenge was found to be decreased even at the lowest-effect concentration of both di- and triorganotin compounds.

- Friedman GC, Hartwick RW, Ro JY, Saleh GY, Tarrand JJ, Ayala AG.
Allergic fungal sinusitis. Report of three cases associated with dematiaceous fungi. Am J Clin Pathol 96:368-372, 1991.
Abstract : Most reported cases of allergic sinusitis have been attributed to Aspergillus, based on the morphologic features of the organisms in tissue sections. However, in most cases, cultures have not been done. This is a report of three cases of non-Aspergillus allergic fungal sinusitis. The patients' ages were 11, 16, and 43; two were male and one was female. Histopathologic study disclosed fungal organisms resembling Aspergillus. However, cultures of these patients' nasal secretions grew Drechslera, Exserohilum, and Bipolaris fungal organisms. The non-Aspergillus nature of these infections was further supported by positive Fontana-Masson melanin staining. The authors conclude that allergic fungal sinusitis most likely results from non-Aspergillus organisms. For definitive fungal identification, tissue culture is mandatory. When tissue is not cultured or no organisms grow, a Fontana-Masson stain can be a useful adjunct in fungal identification.

- Held T, Kutzner HJ.
Formation of melanin in Streptomyces michiganensis (DSM 40 015). DECHEMA Biotechnol Conf

4:353-356, 1990.
Abstract : Melanin is synthesized by the enzyme tyrosinase. A model of tyrosinase formation in S. michiganensis is proposed.

- Jacques SL, McAuliffe DJ.
The melanosome: threshold temperature for explosive vaporization and internal absorption coefficient during pulsed laser irradiation. Photochem Photobiol 53:769-775, 1991.
Abstract : The explosive vaporization of melanosomes in situ in skin during pulsed laser irradiation (pulse duration less than 1 microsecond) is observed as a visible whitening of the superficial epidermal layer due to stratum corneum disruption. In this study, the ruby laser (694 nm) was used to determine the threshold radiant exposure, H0 (J/cm2), required to elicit whitening for in vitro black (Negroid) human skin samples which were pre-equilibrated at an initial temperature, Ti, of 0, 20, or 50 degrees C. A plot of H0 vs Ti yields a straight line whose x-intercept indicates the threshold temperature of explosive vaporization to be 112 +/- 7 degrees C (SD, N = 3). The slope, delta H0/delta Ti, specifies the internal absorption coefficient, mua, within the melanosome: mua = -rho C/(slope(1 + 7.1 Rd)), where rho C is the product of density and specific heat, and Rd is the total diffuse reflectance from the skin. A summary of the absorption spectrum (mua) for the melanosome interior (351-1064 nm) is presented based on H0 data from this study and the literature. The in vivo absorption spectrum (380-820 nm) for human epidermal melanin was measured by an optical fiber spectrophotometer and is compared with the melanosome spectrum.

- Moghadam BK, Gier RE.
Melanin pigmentation disorders of the skin and oral mucosa. Compendium 12:14, 1991.
Abstract : Pigmented lesions in brown, blue-black, or variations of these colors are relatively rare in the oral cavity but very common in the skin and can range from absolutely benign to highly malignant. The differential diagnosis of brown and blue-black lesions of the oral cavity includes normal racial pigmentation, melanosis, nevi, melanoma, amalgam tattoos, and disorders related to the blood or blood vessels.

- Nagaishi H, Oshima N.
Neural control of motile activity of light-sensitive iridophores in the neon tetra. Pigm Cell Res 2:485-492, 1989.
Abstract : Expts. with skin pieces revealed that the sympathetic nervous system controls the activity of the light-sensitive iridophores in the stripes of the neon tetra. The spectral peak reflected from the cells was shifted toward longer wavelengths as a result of a direct interaction between norepinephrine and alpha-adrenoceptors present on the cell membrane. Adenosine accelerated the recovery from the effects of the amine. Such regulation seems to operate when fish are in an excited state or under stress. Since alpha-melanophore- stimulating hormone, melanin-concg. hormone, and melatonin caused the responses only at high concns., it is possible that these peptides and amine do not affect the properties of the light-reflecting cells in vivo.

- Nikolaev ON, Aver'yanov AA.
Effect of passages on artificial nutrient medium on antioxidative properties and pathogenicity of the fungus Pyricularia oryzae Cav. Mikrobiol Zh (Kiev) 53:44-49, 1991.
Abstract : Being subjected to long-term passages on artificial nutrient medium, the culture of the fungus P. oryzae (rice pyriculariosis agent) partially loses its pathogenicity. At the same time, the activity of at least three antioxidative systems (melanin, superoxide dismutase and catalase) falls, which promotes an increase of P. oryzae spore sensitivity to the oxidative damage usually accompanying parasitism and is probably responsible for the extinction of pathogenicity. It is supposed that the development of fungicides selectively suppressing fungal melaninogenesis or other biol. systems of protection from active oxygen forms and thus sensitization the parasite to photodestruction and immune reactions of the host-plant will be one of the directions in control of the plant diseases.

- Thody AJ, Higgins EM, Wakamatsu K, Ito S, Burchill SA, Marks JM.
Pheomelanin as well as eumelanin is present in human epidermis. J Invest Dermatol 97:340-344, 1991.
Abstract : There are two types of melanin in mammals, the brownish black eumelanin and the reddish yellow pheomelanin. Eumelanin and pheomelanin are present in human hair and this study was carried out to see whether both pigments are also present in human epidermis. Samples of epidermis were obtained from suction blisters raised in the upper arm of 13 Caucasian subjects of skin types I, II, and III and analyzed for both eumelanin and pheomelanin using a procedure involving high-performance liquid chromatography. Eumelanin and pheomelanin were found in all epidermal samples and their relative proportions correlated well with those found in samples of hair taken from the same subjects. The lowest concentrations of eumelanin were found in subjects of skin type I, with higher levels in skin types II and III. The concentrations of pheomelanin were more variable and showed no relationship to skin type. Increases in the concentrations of both pigments occurred following PUVA therapy, but whereas the largest increases in eumelanin were seen in skin types II and III, the increases in pheomelanin showed little relationship to skin type. Unlike eumelanin, epidermal pheomelanin also showed little relationship to PUVA-induced tanning. The present findings could be particularly significant in view of recent suggestions that pheomelanin, rather than protecting the skin against UV radiation, may actually contribute to UV-induced skin damage.

- Valentijn JA, Louiset E, Vaudry H, Cazin L.
Dopamine-induced inhibition of action potentials in cultured frog pituitary melanotrophs is mediated through activation of potassium channels and inhibition of calcium and sodium channels. Neuroscience 42:29-39, 1991.
Abstract : A patch-clamp study was conducted in order to investigate the effects of dopamine on the ionic currents in cultured frog melanotrophs. Brief applications of dopamine (1 microM) hyperpolarized the cell and inhibited the spontaneous action potentials. The hyperpolarization was accompanied by an increase in membrane conductance. Under voltage clamp, dopamine evoked a net outward current. The dopamine-induced outward current was negligible at the equilibrium potential for potassium ions. It was also observed that dopamine increased the intensity of a voltage-dependent outward potassium current monitored by constant depolarizing pulses. In addition, voltage-dependent L- and N-like calcium currents and sodium current were reduced. In the cell-attached configuration, two distinct channel types were activated and one channel type was blocked by dopamine exposure to the extrapatch membrane, which indicates the involvement of an intracellular factor in the signal transduction pathway. A higher conductance channel (100 pS) was characterized by a very low basal activity which rapidly increased upon dopamine application. A lower conductance channel (30 pS) displayed a basal activity with frequent opening events, and a delayed (30-40 s) increase of activity in response to dopamine. Both currents reversed at a deduced potential corresponding to the equilibrium potential for potassium ions. The channel type inhibited by dopamine had a low conductance of 15 pS. The inhibition of the electrical activity induced by dopamine was totally blocked by the D2 receptor antagonist S(-)-sulpiride (1 microM) but was not affected by the D1 receptor antagonist SKF-83566 (1 microM). It is concluded that dopamine activates potassium channels and inhibits calcium and sodium channels in frog melanotrophs. The results also indicate that stimulus-response coupling is mediated by intracellular messenger system(s).

- Wollina U, Funfstuck V.
Chloroquine-induced isolated palatal hyperpigmentation. Dtsch Z Mund Kiefer Gesichtschir 14:104-105, 1990.
Abstract : We report about an isolated slate-greyish discoloration of the palatinum during chloroquine therapy of cutaneous lupus erythematosus of a 28-year-old woman. The hyperpigmentation is harmless. It is due to increased melanin synthesis and not due to deposition of the drug or its metabolites. The oculist should be consulted however, to exclude possible retinopathia.

3. MSH, MCH, other hormones, differentiation

- Bodi J, Medzihradszky-Schweiger H, Suli-Vargha H.
Synthesis and biological activity of cyclic melanotropin peptides. Pept. 1990, Proc. Eur. Pept. Symp., 21st, Meeting Date 1990, 690-1. Edited by: Giralt, Ernest; Andreu, David. ESCOM Sci. Publ.: Leiden, Neth., 1991.
Abstract : A report from a symposium on the prepn. and melanin dispersing activity of hexapeptides H-X-His-X1-Arg-Trp-Gly-OMe (I; X = Glu, Gly, X1 = Phe, D-Phe) and cyclopeptides cyclo(Gly-His-X1--Arg-Trp-Gly) (II; X1 = same). I (X1 = D-Phe) were 2 orders of magnitude more potent than I (X1 = Phe), while I (X = Glu) and I (X = Gly) had nearly equal potencies. Cyclization of I (X = Gly) to II resulted in reduced potency.

- Yehuda S, Carasso RL, Mostofsky DI.
The facilitative effects of .alpha.-MSH and melanin on learning, thermoregulation, and pain in neonatal MSG-treated rats. Peptides (Fayetteville, N. Y.) 12:465-469, 1991.
Abstract : Administration of monosodium glutamate (MSG) in the neonatal period renders the rat to be .alpha.-MSH deficient later in life. In this study rats received MSG in their neonatal period and were examd. at the age of 60 days. .alpha.-MSH caused hypothermia, potentiated induced hypothermia, blocked paradoxical behavioral thermoregulation, improved performance in the Morris water tank, but had no effect on pain threshold. Melanin only caused an increase in pain threshold. It is suggested that the differential effect of .alpha.-MSH and melanin is governed by the dopaminergic system.

4. Photobiology and photochemistry

- Andersen PH, Bjerring P.
Noninvasive computerized analysis of skin chromophores in vivo by reflectance spectroscopy. Photodermatol Photoimmunol Photomed 7:249-257, 1990.
Abstract : Reflectance spectroscopy in an objective and accurate method for determining skin colour and has been widely used for measuring physiological variations in skin colour and for monitoring dermatological treatment modalities. Previous studies have used pigment indexes to describe changes in skin colour. Using a multiple regression method to calculate reflectance spectroscopic data, it has been possible to calculate the relative amounts of the different chromophores present in the skin. The technique was found reliable in in vitro tests and in experimentally induced variations in pigment content caused by venous congestion or ultraviolet (UV) light irradiation. In developing UV-induced erythema, the primary events seem to be venous dilatation followed by an increase in blood flow.

- Fuchs J, Packer L.
Electron paramagnetic resonance in dermatologic research with particular reference to photodermatology. Photodermatol Photoimmunol Photomed 7:229-232, 1990.
Abstract : The study of electron paramagnetic resonance (EPR) spectra in isolated skin cells, skin biopsies and the intact skin is the most direct approach to determining the existence, role and importance of ultraviolet-mediated generation of free radicals and other reactive oxygen species in dermatopathological processes. By means of spin labeling, the physicochemical properties of skin, such as membrane fluidity and polarity, can be analyzed. Spin probes can also be employed in measuring one-electron transfer reactions, and oxygen concentration in skin. EPR imaging is an emerging new technique and can be used to investigate the spatial distribution of all these parameters. The more widespread application of the EPR method in photodermatologic research will significantly contribute to improve understanding of biologic free radical processes.

5. Neuromelanins

- Gertz HJ, Schmidt LG.
Low melanin content of substantia nigra in a case of neuroleptic malignant syndrome. Pharmacopsychiatry 24:93-95, 1991.
Abstract : A 19-year old man suffering from a first episode of schizophrenia developed a neuroleptic malignant syndrome (NMS) after administration of haloperidol and levomepromazine. After five weeks of neuroleptic treatment he died of an unknown cause. Histological examination of the brain revealed a low melanin content in neurons in the substantia nigra (SN). Since neuromelanin in SN is the end-product of nonenzymatic dopamine degradation, the amount of melanin probably depends on the overall amount of dopamine produced during life. Thus, dopamine production must have been low in the reported case. In addition, ectopic neurons were found in subcortical white matter.

- Gibb WR, Lees AJ.
Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 54:388-396, 1991.
Abstract : In six control subjects pars compacta nerve cells in the ventrolateral substantia nigra had a lower melanin content than nerve cells in the dorsomedial region. This coincides with a natural anatomical division into ventral and dorsal tiers, which represent functionally distinct populations. In six cases of Parkinson's disease (PD) the ventral tier showed very few surviving nerve cells compared with preservation of cells in the dorsal tier. In 13 subjects without PD, but with nigral Lewy bodies and cell loss, the degenerative process started in the ventral tier, and spread to the dorsal tier. This pattern of selective degeneration of nigrostriatal neurons is not seen in ageing or after acute administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).

- Goto S, Hirano A.
Catecholaminergic neurons in the parabrachial nucleus of normal individuals and patients with idiopathic Parkinson's disease. Ann Neurol 30:192-196, 1991.
Abstract : The parabrachial nucleus is believed to play a role in autonomic regulation. We have used the Fontana-Masson ammoniacal silver nitrate method and a tyrosine hydroxylase-immunostaining technique to demonstrate the presence of neuromelanin-containing catecholaminergic neurons in the parabrachial nucleus of normal individuals. In addition, we also show that there is a significant reduction of these catecholaminergic neurons and presence of Lewy bodies in the parabrachial nucleus of patients with idiopathic Parkinson's disease. These findings may be related to the several autonomic disturbances that may occur in idiopathic Parkinson's disease.

- Inoue K.
A morphological and biochemical study of melanin in the inner ear. Tokyo Joshi Ika Daigaku Zasshi 61:430-438, 1991.
Abstract : The distribution and significance of melanin in the inner ear were compared in humans, guinea pigs, frogs, and fish from the aspect of phylogenesis. Melanin in the membranous labyrinth of guinea pig was analyzed biochem. to det. the properties of melanin and its influence on the inner ear function. In human, melanin was found mostly in the modiolus and the medial vestibular wall of the cochlear duct, followed by the osseous spiral lamina, stria vascularis ductus cochlearis, utricle, the inferior part of the dark cell of the ampulla and semicircular ducts, in that order. In guinea pig, large amts. of pigment were present in the ampulla and in the vicinity of the statoliths of utricle, showing a bar-like form unlike that in frog and fish. In guinea pig melanin was present in the epithelium of the ampulla as in human, and was contiguous with the endolymphatic sac. In the membranous labyrinth of the frog and the fish, a large amt. of melanin was present in the commoncrus of the membranous semicircular canal and ampulla, showing many dendriform processes. Melanin was present in a wide area between the membranous labyrinth and epithelium in the ampulla. These findings did not confirm the theory that melanin acts directly on the inner ear to cause catecholamine in melanosomes in the melanocytes to be released around them.

- Lundqvist M, Arnberg H, Candell J, Malmgren M, Wilander E, Grimelius L, Oeberg K.
Silver stains for identification of neuroendocrine cells. A study of the chemical background. Histochem J 22:615-623, 1990.
Abstract : The chem. background of Ag stains used for visualization and characterization of peripheral neuroendocrine cells in the gastrointestinal tract and pancreas, and of their corresponding tumors, was studied in tissue sections and by a dot-blot technique. Sequential staining of pancreatic islets with an immunohistochem. procedure and Ag staining of the same tissue section revealed that chromogranin A-immunostained cells also displayed an argyrophil reaction with the Grimelius method but no argentaffin reaction with the Masson technique. Accordingly, purified chromogranin A (.ltoreq.15 .mu.g) treated in formalin and applied to nitrocellulose did not show any argentaffin reaction but displayed a dose-related argyrophil reaction. Equal quantities of other polypeptide components did not give rise to any Ag reaction. Other dot-blot studies showed that the tryptophan and tyrosine metabolites dopamine, norepinephrine, 5-hydroxytryptamine, and 5-hydroxyindole caused strongly argentaffin and argyrophil reactions while epinephrine, 5-hydroxyindole-3-acetic acid, and 5-hydroxytryptophan gave only the former reaction. Among other chem. components studied, only guanine displayed weak Ag staining. The results indicate that the reaction products between aldehydes and the granular content of biogenic amines synthesized from tryptophan and tyrosine display an argentaffin reaction and that the granular chromogranin A caused an argyrophil but not argentaffin reaction.

- Saper CB, Sorrentino DM, German DC, de Lacalle S.
Medullary catecholaminergic neurons in the normal human brain and in Parkinson's disease. Ann Neurol 29:577-584, 1991.
Abstract : Parkinson's disease is thought to cause degeneration of melanin-pigmented catecholaminergic neurons throughout the brainstem, but little quantitative information is available on the fate of catecholaminergic neurons associated with the dorsal vagal complex or medullary reticular formation. We therefore examined these neurons in the normal human medulla and in the brains of patients with Parkinson's disease, using both a melanin stain and immunohistochemical methods with an antiserum against tyrosine hydroxylase. The greatest numbers of catecholaminergic neurons in the ventrolateral reticular formation (A1/C1 group) were located in the far rostral medulla, whereas the largest populations of catecholaminergic cells in the dorsal vagal complex (A2/C2 group) were found at the level of the area postrema. No loss of cells was observed in the A1/C1 group in the parkinsonian brains. In contrast, the A2/C2 group showed moderate loss of neurons, most marked at the level of the area postrema. This difference was entirely due to the loss of neurons in the medial component of the A2 group, a population that normally is only lightly pigmented, while the heavily pigmented neurons in the ventral and intermediate components of the A2 complex were unaffected. Parkinson's disease causes degeneration only of selected populations of medullary catecholaminergic neurons, without apparent relationship to the extent of melanin pigmentation.

6. Genetics

- Burchill SA, Ito S, Thody AJ.
Tyrosinase expression and its relationship to eumelanin and pheomelanin synthesis in human hair follicles. J Dermatol Sci 2:281-286, 1991.
Abstract : Tyrosinase expression was examd. in hair follicles from red- and dark-haired individuals. Tyrosinase activity was greater in the hair follicles of red-haired subjects than in those from dark-haired subjects. Tyrosinase synthesis (as detd. by incorporation of label and immunopptn.) was also greater in the red-haired subjects and this presumably accounted for their higher levels of tyrosinase activity. The levels of tyrosinase synthesis in the red-haired subjects correlated well with the pheomelanin content in the hair, but in the dark-haired individuals a better correlation was seen with emumelanin. .alpha.-MSH failed to increase tyrosinase synthesis in the hair follicles of either group of subjects and in the follicles from the redheads actually produced a decrease. On the other hand, 8-bromo-cAMP increased tyrosinase synthesis but only in the hair follicles from dark-haired subjects. These findings contrast with those previously reported in mice, and apparently the control

mechanisms that regulate tyrosinase in human melanocytes are different in many respects from those in mice.

- Hawkins FKL, Kennedy C, Johnston AWB.
A Rhizobium leguminosarum gene required for symbiotic nitrogen fixation, melanin synthesis and normal growth on certain growth media. J Gen Microbiol 137:1721-1728, 1991.
Abstract : The gene nfrX in Azotobacter vinelandii activates transcription of other nif genes in that species. A cosmid contg. cloned R. leguminosarum DNA that cor. the Nif- defect of an nfrX mutant of A. vinelandii was isolated. Following Tn5 transposon mutagenesis of the cosmid in Escherichia coli, mutant derivs. unable to correct the A. vinelandii nfxX mutants were obtained in 2 sep. regions of DNA. In addn., mutations close to one of the nfrX regions conferred a complex phenotype when introduced into the Rhizobium genome by marker exchange. These mutants induced non-fixing nodules on peas, were slow-growing on media with succinate as C source or nitrate as N source and, when present in R. leguminosarum biovar phaseoli, they failed to make melanin, a pigment that is normally synthesized by R.l. bv. phaseoli. The mutated gene, termed melC, was fused to uidA (which encodes .beta.-glucuronidase); transcription of melC-uidA was enhanced in microaerobic conditions and it was expressed at high levels in infection threads in pea nodules.

- Held T, Kutzner HJ.
Genetic recombination in Streptomyces michiganensis DSM 40,015 revealed three genes responsible for the formation of melanin. J Basic Microbiol 31:127-134, 1991.
Abstract : By UV and NTG mutagenesis numerous auxotrophic, antibiotic-resistant and melanin-negative mutants were isolated from Streptomyces michiganensis DSM 40,015 which proved to possess a efficient photoreactivation system. Using a mel- test strain with three auxotrophic markers and a antibiotic resistance for crosses with numerous prototrophic mel- strains three classes of mutants (melA, melB and melC) could be found. This classification was further supported by a series of crosses. The melC locus seemed to correspond to the melC locus of S. glaucescens which contains the tyrosinase structural gene.

- L'Helias C.
Hormonal imbalance by pteridine treatment in diapausing Pieridae induces mutations in the progeny of treated Drosophila melanogaster. Chem Biol Pteridines, 1989 Proc Int Symp Pteridines Folic Acid Deriv, 9th, Meeting Date 1989, 462-5. Edited by: Curtius, Hans-Christoph; Ghisla, Sandro; Blau, Nenad. de Gruyter: Berlin, Fed Rep Ger, 1990.
Abstract : In the previously described expts., the disorders which appear at the somatic level reveal a const. relation between the pterins and melanization melanins and ommochromes, sclerotinization, gonadal control, and cellular differentiation control. A disorganization can then induce cellular proliferation. In these expts., there is still non abs. proof that the action of pterins is sited at the level of modulation of hormonal control by the photoreceptor center.

- O'Donnell JM, McLean JR.
Molecular characterization of the GTP cyclohydrolase I gene of Drosophila: analysis of cDNA clones. Chem Biol Pteridines, 1989 Proc Int Symp Pteridines Folic Acid Deriv, 9th Meeting Date 1989, 316-19. Edited by: Curtius, Hans-Christoph; Ghisla, Sandro; Blau, Nenad. de Gruyter: Berlin, Fed Rep Ger, 1990.
Abstract : GTP cyclohydrolase I (GTPCH) catalyzes the first step in pteridine biosynthesis. In Drosophila melanogaster this enzyme is encoded by a genetic locus called Punch (Pu). Mutations in Pu often lead to biochem. alterations in GTPCH, including thermal lability, variant isoforms and redns. in GTPCH protein and activity levels (1-4). These alterations can generally be correlated with effects on two Drosophila functions that require pteridines. These are eye pigmentation and the prodn. of catecholamines which, in Drosophila, are used as neurotransmitters, melanin precursors and cuticle cross-linkers. The authors investigated the relationship between the complex genetics of Pu, the biochem. of pteridine biosynthesis and function, and the mol. organization of the region. They found that the Pu locus also appears to be assocd. with requirements for pterin cofactors. In addn., the locus expresses numerous transcripts, at least two of which overlap extensively in sequence as

defined by cDNA anal. These transcripts are expressed at times of known functions and are altered in Pu mutants.

- Pentz ES, Wright TR.
Drosophila melanogaster diphenol oxidase A2: gene structure and homology with the mouse mast-cell tum- transplantation antigen, P91A. Gene 103:239-242, 1991.
Abstract : The Drosophila melanogaster diphenol oxidase (DOX) A2-encoding gene (Dox-A2) is involved in catecholamine metabolism, melanin formation and sclerotization of the cuticle. Insect phenol oxidases (POX) are well studied biochemically, but not genetically and molecularly. The Dox-A2 (2-53.9) gene is the first insect POX-encoding gene to be cloned and sequenced. It encodes a protein product unique among currently known POX. The deduced protein, however, exhibits extensive similarity (58-81%) to the mouse mast cell tum- antigen, P91A [Lurquin et al., Cell 58 (1989) 293-303] and may identify the normal mouse protein as a DOX.

- Shibahara S, Taguchi H, Muller RM, Shibata K, Cohen T, Tomita Y, Tagami H.
Structural organization of the pigment cell-specific gene located at the brown locus in mouse. Its promoter activity and alternatively spliced transcript. J Biol Chem 266:15895-15901, 1991.
Abstract : The pigment cell-specific gene, located at the brown (b) locus in mouse, has been cloned and characterized. Its gene product is required for the formation of black melanin rather than brown, although its exact function remains to be elucidated. We thus tentatively named it b-locus protein in this report. The b-locus protein gene is about 18 kilobase pairs long and organized into 8 exons and 7 introns. Functional analysis of its promoter region suggests that the nucleotide residues -38/154 is sufficient to direct the pigment cell-specific transcription in melanoma whole cell extracts. On the other hand, we were unable to detect its transcripts in HeLa whole cell extracts. Sequence comparison with the promoter region of the tyrosinase gene, another pigment cell-specific gene, reveals that two elements of the b-locus protein gene (-33/-24 and 18/28) are also conserved in the tyrosinase gene at equivalent positions, suggesting that these two elements may be involved in their pigment cell-specific transcription. Furthermore, we have cloned a cDNA, pMT3, coding for an isoform of b-locus protein from a cDNA library of mouse B16 melanoma cells. Sequence analysis of pMT3 reveals a deletion of 103 base pairs, which corresponds to the 5'-end of the exon 8 of the b-locus protein gene, indicating that pMT3 represents a mRNA species generated by alternative splicing. Since this deletion changes the reading frame and eliminates the transmembrane domain of b-locus protein, the pMT3-type mRNA may code for a soluble isoform. Such an isoform, consisting of 553 amino acids, differs only in its carboxyl terminus and is larger than b-locus protein by 16 amino acids. Using transient expression assays, we confirmed that such an isoform is able to react with anti-b-locus protein monoclonal antibody, TMH-1, suggesting that a b-locus protein isoform may have some function in pigmentation.

- Silversides FG, Crawford RD.
Phenotypic, embryonic, and neonatal effects of a gene for sex-linked imperfect albinism (Sal-s) in chickens. Poult Sci 70:1306-1313, 1991.
Abstract : Gross phenotypic observations, histology, and tissue culture showed that the gene for sex-linked imperfect albinism that occurred at the University of Saskatchewan (Sal-s), allows a small amount of melanin pigment to be deposited in eyes and feathers. Melanin pigment accumulates in retinal pigment epithelial and cultured neural crest cells, but neural crest cells pigmenting the feathers transfer their pigment as it is produced, and this is seen as a constant amount of color in successive generations of feathers. Despite differences from early reports, it would appear that the phenotype produced by Sal-s is essentially the same as that produced by other Sal mutations. Albinos have a high incidence of lesions in the regions of the navel, the hocks, and the nares, similar to those associated with other hypomelanic mutations in the chicken. Yolk contents appear to be used more slowly by albinos late in incubation. The increased size of the yolk sacs probably contributes directly to producing the navel lesions and indirectly to variation in hatch weight. Albinos have small bursae of Fabricius, reduced hatchability, and early growth.

- Walter MF, Black BC, Afshar G, Kermabon AY, Wright TR, Biessmann H.
Temporal and spatial expression of the yellow gene in correlation with cuticle formation and dopa decarboxylase activity in Drosophila development. Dev Biol 147:32-45, 1991.
Abstract : The yellow (y) gene of Drosophila is required for the formation of black melanin and its deposition in the cuticle. We have studied by immunohistochemical methods the temporal and spatial distribution of the protein product of the y gene during embryonic and pupal development and have correlated its expression with events of cuticle synthesis by the epidermal cells and with cuticle sclerotization. Except for expression in early embryos, the y protein is only found in the epidermal cells and may be secreted into the cuticle as it is being deposited. The amount of y protein in various regions of the embryo and pupa correlates directly with the intensity of melanization over any section of the epidermis. Expression of the y gene begins in the epidermal cells at 48 hr after pupariation and is well correlated with the beginning deposition of the adult cuticle. At this stage the adult cuticle is unsclerotized and unpigmented and dopa decarboxylase levels, a key enzyme in catecholamine metabolism which provides the crosslinking agents as well as the precursors for melanin, is low. As a separate event 26 hr after the onset of y gene expression, the first melanin deposition occurs in the head bristles and pigmentation continues in an anterior to posterior progression until eclosion. This melanization wave is correlated with elevated dopa decarboxylase activity. Crosslinking of the adult cuticle also occurs in a similar anterior to posterior progression at about the same time. We have shown by imaginal disc transplantation that timing of cuticle sclerotization depends on the position of the tissue along the anterior-posterior axis and that it is not an inherent feature of the discs themselves. We suggest that actual melanization and sclerotization of the cuticle by crosslinking are initiated at this time in pupal development by the availability of the catecholamine substrates which diffuse into the cuticle. Intensity of melanization and position of melanin pigment is determined by the presence or absence of the y protein in the cuticle, thus converting the y protein prepattern into the melanization pattern.

- Winder AJ.
Expression of a mouse tyrosinase cDNA in 3T3 Swiss mouse fibroblasts. Biochem Biophys Res Commun 178:739-745, 1991.
Abstract : 3T3 Swiss mouse fibroblast cell lines expressing tyrosinase, the critical enzyme in melanin synthesis, have been established by co-transfection of a mouse tyrosinase cDNA and a G418-resistance gene. Of sixty-three clones isolated, four are brown in colour, presumably due to synthesis of melanin. Expression of both the tyrosine hydroxylase and dopa oxidase activities of tyrosinase by these pigmented clones has been demonstrated directly by enzyme assays. Electron microscopic studies suggest that the brown pigment is located in membrane-bound cytoplasmic vesicles.

- Yada Y, Higuchi K, Imokawa G.
Effects of endothelins on signal transduction and proliferation in human melanocytes. J Biol Chem 266:18352-18357, 1991.
Abstract : Human melanocytes are regulated by endothelin (ET) derivs., potent vasoconstrictive peptides synthesized by endothelial cells, to stimulate their proliferation and melanization via a receptor-mediated signal transduction pathway. Receptor-binding assay using [125I]ET indicated that unlabeled ET-1 or ET-2 competitively inhibited each binding of labeled ETs to melanocytes with a concn. for half-maximal inhibition (IC50) of 0.7 or 0.9 nM, resp. The dissocn. const. (Kd) and the no. of sites of the specific bindings of ET-1 and those of ET-2 were almost the same (Kd: 1.81 nM, binding sites: 7.0-8.0 .times. 104 per cell). Upon incubation with cultured cells, the mass contents of inositol 1,4,5-trisphosphate and intracellular Ca level were substantially increased by 10 nM ET-1, ET-2, and ET-3, but not by big-Et with maximal response at 80-130-s postincubation. The addn. of ET-1 and ET-2 at 1-50 nM concns. caused human melanocytes to stimulate DNA ([3H]thymidine incorporation) and melanin synthesis (3H2O release and [14C] thioruacil incorporation). Furthermore, ETs exhibited an additive stimulatory effect on basic fibroblast growth factor-stimulated DNA synthesis. In a long-term serum-free culture system, the strongest stimulation of growth by 10 nM ET-1 or ET-2 was obsd. in the presence of 10 nM cholera toxin and 0.2% bovine pituitary ext., resulting in a 4.5-fold increase in cell no. for 12 culture days. These findings strongly suggest involvement of ET in the mechanism regulating proliferation and melanization of human

melanocytes.

7. Tyrosinase and other enzymes

- Aroca P, Solano F, Garcia-Borron JC, Lozano JA.
Specificity of dopachrome tautomerase and inhibition by carboxylated indoles. Considerations on the enzyme active site. Biochem J 277:393-397, 1991.
Abstract : Dopachrome tautomerase (EC 5.3.2.3) catalyses the tautomerization of dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) within the melanin-formation pathway. We have analysed a series of substrate analogues and related compounds as possible substrates and inhibitors of tautomerization. The enzyme appears to be highly specific since D-dopachrome, alpha-methyldopachrome, dopaminochrome, adrenochrome methyl ether and deoxyadrenochrome are not substrates. Conversely, dopachrome tautomerase catalyses the tautomerization of dopachrome methyl ester, suggesting that a carboxy group, either free or as a methyl ester, is essential for enzyme recognition. No inhibition of dopachrome tautomerization was observed in the presence of either semiquinonic compounds, such as tropolone and L-mimosine, or pyrrole-2-carboxylic acid and unsubstituted indole. However, a number of indole derivatives, including DHICA, the product of dopachrome tautomerization, and the analogues 5-hydroxyindole-2-carboxylic and indole-2-carboxylic acid were able to inhibit the enzyme. Furthermore, indoles with a side chain at position 3 of the ring and containing a carboxylic group at the gamma-position of this chain, such as L-tryptophan or indole-3-propionic acid, are stronger inhibitors of the enzyme. Indole-3-carboxylic acid, indol-3-acetic acid and indole-3-butyric acid are very weak inhibitors, showing that the carboxylic group needs to be located at an optimal distance from the indole ring to mimic the carboxylic group at position 2 on the authentic substrate.

- Rachkova M, Raikova E, Raikov Z.
New nitrosoureas and their spin-labeled derivatives influence DOPA-oxidase activity of tyrosinase. Cancer Biochem Biophys 12:59-64, 1991.
Abstract : Tyrosinase is a key enzyme in melanin biosynthesis. The modulating effect of cytostatic agents on DOPA-oxidase activity of tyrosinase could be linked with the drug treatment of melanoma tumors. Two groups of nitrosoureas which influence DOPA-oxidase activity of tyrosinase were studied: new nitrosoureas and their spin-labeled derivs. synthesized in the authors' lab. Using Burnett's spectrophotometric method, the following effects were established: inhibition by CCNU, inhibition and the activating effects of the other investigated nitrosoureas depend on their physicochem. half-life. The predominant activating effect of the spin-labeled derivs. is due to the nitroxyl radical present in these compds.

8. Melanoma

- Akamatsu H, Komura J, Asada Y, Miyachi Y, Niwa Y.
Inhibitory effect of azelaic acid on neutrophil functions: a possible cause for its efficacy in treating pathogenetically unrelated diseases. Arch Dermatol Res 283:162-166, 1991.
Abstract : It has been shown that acne, hyperpigmentation and lentigo malignant are more or less related pathogenetically to reactive oxygen species (ROS). It has recently been reported that azelaic acid is effective in treating these conditions and that it possesses anti-enzymatic and anti-mitochondrial activity, including cytochrome-P450 reductase and 5 alpha-reductase in microsomal preparations with nicotinamide adenine dinucleotide phosphate (NADPH). We therefore investigated the effects of azelaic acid on human neutrophil functions, such as chemotaxis, phagocytosis and ROS generation. ROS generation in a cell-free system was also assessed. The results revealed that neutrophil chemotaxis and phagocytosis as well as ROS generated in a xanthine-xanthine-oxidase system were not significantly changed in the presence of azelaic acid. However, azelaic acid markedly decreased O2- and OH. generated by neutrophils. It may be concluded that the reported clinical effectiveness of azelaic acid is partly due to its inhibitory action on neutrophil-generated ROS, leading

to a reduction both in oxidative tissue injury at sites of inflammation and in melanin formation.

- Bustamante J, Guerra L, Bredeston L, Mordoh J, Boveris A.
Melanin content and hydroperoxide metabolism in human melanoma cells. Exp Cell Res 196:172-176, 1991.
Abstract : Human melanoma cells were grown to exponential and stationary phases showing melanin contents of 4.2 +/- 0.3 and 11.3 +/- 0.6 micrograms/10(6) cells, respectively. The cells were separated in four subpopulations by a Percoll gradient; the subpopulation of density 1.07 (g/ml) was the most enriched in pigmented cells and produced 28 and 58% of the cells in exponential and stationary phases, respectively. Melanoma cells had similar superoxide dismutase and glutathione peroxidase activities in exponential and stationary phases. Moreover melanoma cells exhibited a higher catalase activity in the stationary phase: whole homogenate and cytosol activities were 7.0 +/- 0.3 and 10.8 +/- 0.6 U/mg protein, whereas in exponential phase the activities were 4.9 +/- 0.1 and 7.6 +/- 0.3 U/mg protein for whole homogenate and cytosol, respectively. The intracellular H2O2 steady-state concentration was 3.3 +/- 0.2 and 2.1 +/- 0.2 microM H2O2 for exponential and stationary phases, respectively. The spontaneous chemiluminescence of the two culture phases was 169 +/- 27 cps/10(6) cells (exponential) and 78 +/- 24 cps/10(6) cells (stationary). The cytotoxicity of H2O2 generated extracellularly by glucose oxidase was determined after 60 min of exposure. IC50 values for exponential and stationary cell cultures were 0.9 and 2.4 mU/ml of glucose oxidase, respectively. The increased catalase activities in the stationary phase as compared with the exponential phase are consistent with the decreased intracellular H2O2, with the decreased spontaneous chemiluminescence, and with the increased resistance to exogenous H2O2.

- Coderre JA, Glass JD, Packer S, Micca P, Greenberg D.
Experimental boron neutron capture therapy for melanoma: systemic delivery of boron to melanotic and amelanotic melanoma. Pigment Cell Res 3:310-318, 1990.
Abstract : The boron-containing melanin precursor analogue p-boronophenylalanine (BPA) has previously been shown to selectively deliver boron to pigmented murine melanomas when administered in a single intragastric dose. If boron neutron capture therapy is to become a clinically useful method of radiation therapy for human malignant melanoma, the boron carrier must be capable of delivering useful amounts of boron to remote tumor sites (metastases) and to poorly pigmented melanomas. We have now determined the ability of BPA to accumulate in several nonpigmented melanoma models including human melanoma xenografts in nude mice. The absolute amount of boron in the nonpigmented melanomas was about 50% of that observed in the pigmented counterparts but was still selectively concentrated in the tumor relative to normal tissues in amounts sufficient for effective neutron capture therapy. Single intragastric doses of BPA resulted in selective localization of boron in the amelanotic Greene melanoma carried in the anterior chamber of the rabbit eye and in a pigmented murine melanoma growing in the lungs. The ratio of the boron concentration in these tumors to the boron concentration in the immediately adjacent normal tissue was in the range of 3:1 to 4:1. These distribution studies support the proposal that boron neutron capture therapy may be useful as a regional therapy for malignant melanoma.

- Kwittken J.
Cutaneous melanocytic dysplasia and malignant melanoma. A critical review. J Med 22:1-16, 1991.
Abstract : Melanocytic dysplasia refers not only to a variety of histologic changes, but is also a histologic diagnosis. Melanocytic dysplasia represents a diagnosis for those lesions in which the biologic potential cannot be determined. Histologic differences between a diagnosis of melanocytic dysplasia and malignant melanoma are detailed. The terms: borderline malignant melanoma or lesion, precancerous melanosis, active or activated nevus and atypical melanocytic hyperplasia should be abandoned. A differential diagnosis between in situ malignant melanoma and other melanin-containing in situ malignancies has been presented. All types of malignant melanoma which originate within the epidermis or epithelium have an in situ phase and show horizontal growth, and small early forms defy classification. The classification of the most common types of malignant melanoma has been revised. Although the level of invasion and tumor thickness are useful prognostic and therapeutic parameters, their determination is crude and subject to several shortcomings.

- Mirich DR, Blaser SI, Harwood-Nash DC, Armstrong DC, Becker LE, Posnick JC.
Melanotic neuroectodermal tumor of infancy: clinical, radiologic, and pathologic findings in five cases. AJNR Am J Neuroradiol 12:689-697, 1991.
Abstract : Five pathologically proved melanotic neuroectodermal tumors of infancy are reported. These rare neoplasms of infancy exhibit a distinct predilection for the maxillary bone. Three tumors originated in the maxilla, one in the calvaria, and one in the cerebellar vermis. Those occurring in bone did not metastasize but were locally invasive, as reflected in their radiologic appearance. Bone erosion, expansion, hyperostosis, and osteogenesis can occur in the same neoplasm and were appreciated best on CT. MR imaging showed the soft tissue component and extent of the neoplasm better than CT did. The pathologic findings from all five cases (and one possibly related melanotic tumor of the face) revealed abundant melanin. MR imaging of two melanotic tumors showed isointense T1-weighted and slightly hyperintense T2-weighted signals. This appearance is contrary to that of most melanin-containing tumors, which exhibit enhanced T1 and T2 relaxation, and indicates that variables other than the absolute amount of melanin may determine the MR signal. Clinically, rapid neoplastic growth and excessive melanin production by the tumor cells caused facial disfigurement and visible blue black discoloration. All five melanotic neuroectodermal tumors were resected and the vermian tumor was also irradiated. Four of five children were well and free from disease 1 month to 7 years after resection. The calvarial tumor was incompletely resected and involved the underlying brain, eventually causing death. The clinical, radiologic, and pathologic features of melanotic neuroectodermal tumors of infancy are reviewed. Melanotic neuroectodermal tumors of infancy that involve bone can be diagnosed from the clinical and radiologic findings. Prompt diagnosis and surgical resection are essential for cure.

- Moragon M, Pascual R, Ferriz P, Carbonell MA, Ribon F.
Diffuse melanosis in metastatic malignant melanoma with melanuria. An Med Interna 7:367-369, 1990.
Abstract : We report an additional case of diffuse melanosis secondary to metastases from malignant melanoma in a patient, who was seen in our department shortly before death. We couldn't localize the origin of the primary neoplasm. After reporting the case, we discuss the pathogenesis of melanosis and possible sites of the primary tumor.

- Nakashima N, Mitsumori K, Maita K, Shirasu Y.
Amelanotic melanocytic tumors of the pinna in six F344 rats. J Vet Med Sci 53:291-296, 1991.
Abstract : Spontaneous amelanotic melanocytic tumors of the pinna were found in six females of 960 male and 960 female albino (F344/DuCrj) rats which had been used in three different 24-month chronic toxicity studies. The age when the pinnal tumors were detected ranged from 37 to 59 weeks. The tumors were located unilaterally in the pinna and observed as subcutaneous spherical to irregular, solid white masses measuring 7 to 25 mm in diameter. The pinnal tumors were histologically classified into spindle cell and pleomorphic cell types. The spindle cell type was observed in four rats and composed of fusiform cells arranged in interlacing bundles. The pleomorphic cell type was observed in the remaining two rats and composed of pleomorphic large cells arranged in sheets. One tumor of the latter type metastasized to the submaxillary lymph node and lung. Melanin pigments were not demonstrated in any of the tumors. In immunohistochemistry, nuclei and cytoplasm of tumor cells in all the tumors were slightly positive for S-100 protein. Ultrastructurally, tumor cells contained a considerable number of premelanosomes in the cytoplasm. Desmosomes were occasionally observed between the cell membranes of the adjacent tumor cells. No distinct basal lamina was seen around tumor cells.

- Pock L, Trnka J, Vosmik F, Zaruba F.
Systematized progradient multiple combined melanocytic and blue nevus. Am J Dermatopathol 13:282-287, 1991.
Abstract : A 44-year-old woman was diagnosed as having unilateral multiple progradient pigmented macules and papules of the upper extremity and adjacent part of the back. Microscopically increased amounts of melanin and melanocytes in the basal layer of the epidermis in the early developmental stage of macules were seen. Combinations of blue nevus with junctional or compound nevus or with

a simple proliferation of melanocytes in the epidermis were present in the papules. The question of prognosis is discussed.

- Schwabe K, Lassmann G, Damerau W, Naundorf H.
Protection of melanoma cells against superoxide radicals by melanins. Malig. Melanom, 41:100-103, 1989.
Abstract : Macrophages, granulocytes, and other effector cells kill tumor cells by releasing highly toxic superoxide radicals. Melanoma cells are characterized by a high content of melanin pigment. Isolated melanin has been shown to be a potent scavenger of superoxide radicals. Perhaps melanoma cells escape from the immunol. attack by quenching cytotoxic superoxide radicals. In order to test this hypothesis, the reaction of superoxide radicals with different melanin prepns. in comparison with normal muscle tissue of the same animal was studied by ESR.

- Umemura T, Ohya Y, Naoi M, Nagatsu T, Fukui Y, Yasue T, Ohashi M.
Selective toxicity of 1-methyl-4-phenylpyridinium ion (MPP+) to pigmented melanoma cells in vitro. Anticancer Drug Des 6:207-210, 1991.

- Welkoborsky HJ, Sorger K, Knuth A, Bernal-Spekrelsen M, Dippold WG.
Malignant melanoma of the mucous membranes of the upper aerodigestive tract. Clinical, histological and immunohistochemical characteristics. Laryngorhinootologie 70:302-306, 1991.
Abstract : Malignant melanomas of the mucous membranes are rare tumors. They make up about 10% of all malignant melanomas of the head and neck; 15 of the authors' cases are reviewed in this article. Six of these had neck lymph node metastasis when first diagnosed. The tumors were surgically removed in all patients. Thirteen patients developed at least one tumor recurrence, ten patients distant metastasis. Eight patients died of the tumor condition; the mean survival time of all patients was 33.4 months. While the tumors could be classified histologically into four types, this had no bearing on the course of the disease. In many cases, primary tumor and metastasis or recurrent tumor differed histologically. Melanin pigment was found in 13 tumors. Mucosal melanomas can be regarded as a discrete tumor entity because their biological behavior differs from that of malignant melanomas of the skin. However there are no morphological differences between the two tumor entities. Ophthalmological and dermatological examinations must be performed in all patients with mucosal melanoma to exclude metastasis of the skin or choroid melanoma.

9. Eye

- Balkema GW, Drager UC.
Impaired visual thresholds in hypopigmented animals. Vis Neurosci 6:577-585, 1991.
Abstract : Ocular hypopigmentation is associated with neurological defects in structure and function. This paper investigates the absolute visual thresholds in dark-adapted hypopigmented animals compared to their normally pigmented controls. Here we asked (1) whether the threshold elevation found in hypopigmented animals is a general consequence of the reduction in melanin content; (2) if so, which melanin components in the eye are likely to influence visual thresholds; and (3) whether similar threshold defects can be detected in orders other than rodents. By single-unit recordings from the superior colliculus, we compared incremental thresholds of normal black mice of the C57BL/6J strain to hypopigmented mutants: beige (bg/bg), pale ear (ep/ep), and albino (c2J/c2J) mice, three mutants in which melanin pigment throughout the body is affected; and Steel (Sl/Sld) and dominant-spotting/W-mice (W/Wv), two mutants with normal pigmentation in the retinal pigment epithelium (RPE) but without any melanin in the choroid or the rest of the body. We found that all mutants had elevated thresholds that varied with the reduction in melanin. The albinos were 25 times less sensitive than black mice, pale ear mice 20 times, beige mice 11 times, and Steel and W-mice 5 times. The mean thresholds of dark-adapted black mice were 0.008 cd/m2. Recordings from rabbits showed a similar impairment of visual sensitivity; incremental thresholds were elevated 40 times in New Zealand-White albino rabbits (0.0008 cd/m2) compared to Dutch-Belted pigmented controls (0.00002 cd/m2).

- Docchio F, Boulton M, Cubeddu R, Ramponi R, Barker PD.
Age-related changes in the fluorescence of melanin and lipofuscin granules of the retinal pigment epithelium: a time-resolved fluorescence spectroscopy study. Photochem Photobiol 54:247-253, 1991.
Abstract : The photophys. properties of purified population of melanin and lipofuscin granules from human retinal pigment epithelium, and their changes with donor age were investigated by using high-sensitivity time-resolved fluorescence spectroscopy techniques with picosecond gating capabilities. The overall fluorescence intensity of both melanin and lipofuscin granules clearly increased with increasing donor age, the increase being most marked for melanin. In all granule populations the fluorescence decays were multiexponential with subnanosecond and nanosecond decay components. The resultant time-integrated and time-gated spectra also exhibited marked age variations for each type of granule. Young melanin showed spectral patterns similar to those of bovine melanin, while a yellow-orange fluorescence band appeared in melanin samples from older age group. Lipofuscin granules exhibited a blue, a yellow, and an orange band whose relative amts. were age-related. The results demonstrate the potential of time-resolved techniques for discriminating fluorophores invitro and in situ, and confirmed results previously obtained by using extn. techniques. Furthermore, the ability of this technique to identify and quantify individual fluorophores within granules may provide an important insight into the origin and development of lipofuscin within the retinal pigment epithelium and ultimately into the mechanisms of age-related retinal diseases.

- Saika S, Tonoe O, Kanagawa R, Uenoyama K, Yamanaka A, Fukuda K, Iwane H.
Immunohistochemical study of deposits on intraocular lenses explanted from human eyes. Jpn J Ophthalmol 35:96-101, 1991.
Abstract : Immunohistochemical studies of deposits were carried out on two intraocular lenses (IOLs) explanted from human eyes. One anterior chamber intraocular lens (AC-IOL) was studied using a monoclonal anti-human type I collagen-peptide antibody (C-Ab). One posterior chamber intraocular lens (PC-IOL) was studied using a monoclonal anti-human vimentin antibody (V-Ab). Most of the cells on the AC-IOL contained many melanin granules in the cytoplasm and were thought to be macrophages. They did not show any immunoreactivity to C-Ab. Some spindle-shaped cells and fibrous deposits at the margin of the lens optics showed immunoreactivity to the antibody. These cells were thought to be fibroblasts migrating from the tissue around the IOL, such as the iris. On the PC-IOL, many mononuclear cells and multinucleated giant cells were observed. These cells showed immunoreactivity to vimentin and contained immunostained fibers which were intermediate filaments. They were thought to be either of mesodermal origin or derived from the lens epithelium.

- Steinmetz RL, Garner A, Maguire JI, Bird AC.
Histopathology of incipient fundus flavimaculatus. Ophthalmology 98:953-956, 1991.
Abstract : A 9-year-old boy was diagnosed with fundus flavimaculatus in his left eye. The boy's fellow eye was enucleated at 16 months of age for retinoblastoma. The authors reviewed the material submitted for histopathologic examination and found that the retinal pigment epithelial cells demonstrated increased autofluorescence and increased reactivity to periodic acid-Schiff staining. Many cells had their melanin granules displaced toward the cell apex. The retinal pigment epithelial changes are consistent with previous histopathologic findings in fundus flavimaculatus and imply that the structural changes are seen in early life.

- Suzuki T, Ohga H, Katayama T, Egi K, Fujiwara H, Mizushima M.
A girl with Hermansky-Pudlak syndrome. Acta Ophthalmol (Copenh) 69:256-260, 1991.
Abstract : A young girl with ocular albinism and the Hermansky-Pudlak syndrome is described. Ocular albinism generally occurs in males. In this condition, the pigmentation of the skin and hair is nearly normal, and the melanin pigment abnormality is limited to the eyeballs. The chief complaints are visual disturbance, nystagmus, and photophobia. A 3-year-old girl was recently brought to our hospital with nystagmus, which she had exhibited since the age of 1 year. Funduscopy resulted in a diagnosis of ocular albinism. Further investigations, specifically, microscopy of her platelets, led us to conclude that she had Hermansky-Pudlak syndrome.

10. Other

- Proca M, Cotrau M, Butnaru E.
  Biotoxicological research on a segment of the population in an industrial environment. I. Total proteins, hemoglobin, methemoglobin and cholinesterase. Rev Med Chir Soc Med Nat Iasi 94:363-367, 1990.
  Abstract : The inevitable chemical risks present in a synthetic threads and fibres aggregate works determine a careful and permanent surveillance of employees' state of health, especially where the occupational risk is known to exist. Besides the usual labour protection measures, screening for the early detection of some biochemical or biotoxicological changes induced by the contact with the chemical noxae in the labour environment are performed. This paper presents the results of a complex biochemical and biotoxicological screening including about 300 employees working at the polyplants in the melanin section where the major chemical noxa is acrylonitrile. The total proteins, hemoglobin, methemoglobin and cholinesterase activity were determined.

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EXTRACTS FROM MURPHY'S LAW

- In order to get a loan, you must first prove you don't need it.
- Everyone has a scheme for getting rich that will not work.
- In a hierarchy, each individual rises to his own level of incompetence, and then remains there.
- If you try to please everybody, nobody will like it.
- When in doubt, mumble. When in trouble delegate.
- Anything good in life is either illegal, immoral of fattening.
- It is morally wrong to allow suckers to keep their money.
- Beauty is only skin-deep, ugly goes to the bone.
- To know yourself is the ultimate form of aggression (Freudian psychology).
- Never play leapfrog with a unicorn.
- If everything seems to be going well, you obviously don't know what the hell is going on.
- In case of doubt, make it sound convincing.
- Never argue with a fool, people might not know the difference.