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CONTENTS

<i>Discussion : Materia melanica: a pigmented perception</i> Riley P.A.	316
<i>Review of the literature</i>	323
1. Melanins and other pigments chemistry	323
2. Biology of pigment cells and pigmentary disorders	325
3. MSH, MCH, other hormones, differentiation	334
4. Photobiology and photochemistry	335
5. Neuromelanins	338
6. Genetics	339
7. Tyrosinase and other enzymes	341
8. Melanoma and other pigmented tumours	344
9. Eye	347
10. Other	350
<i>News from the ESPCR</i>	353
<i>Call for contributions</i>	355
<i>Announcements and related activities</i>	356

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



MATERIA MELANICA: A PIGMENTED PERCEPTION

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On July the 11th 1991 there was a total eclipse of the sun visible from Mexico at midday. It is clear from the enormous corona that this star is an immense source of energy. The primacy of this energy source in sustaining life on this planet was recognized in the Bronze Age - prehistoric solar religion. In particular, the early Egyptian civilization who imbued the sun with divinity: the God Ra riding daily across the sky in his chariot. Although it is 96 million miles away the amount of energy falling on the earth's surface greatly exceeds any other source of energy. It is presently the ultimate source of all energy in the biosphere.

Origin of Life

It seems likely that life actually originated in conditions which permitted the utilization of geochemical sources of energy. The generation of the first self-replicating systems (either proteins or nucleic acids) arose from the "primaevial soup" which, according to Orgel, had the consistency of a well-known packet soup made up to the manufacturers' specification.

Life as Negentropic State

We may define life as an "improbable state of order perpetuated in time". Life is thus an exception to the general time-dependent trend of increasing entropy which is characteristic of our present universe. To sustain this creation of order (anabolic activity) energy is required and this is derived from the catabolism or breakdown of order in other molecules. It is likely that molecules with large energy yields, similar to ATP, existed initially in plentiful supply in the primitive geochemically-generated environment.

Metabolic Coupling: Compartmentation

It is clear that an advantage would accrue to systems that could strongly couple catabolism to anabolic activity and this would result from containment of the reactants. This compartmentation (for example, by membranes which would form spontaneously from amphiphathic molecules such as phospholipids) permits the action of natural selection and therefore the advent of evolution in the sense described by Darwin. It

should be noted that selection cannot operate in an open system - an example of this perhaps provided by economic systems.

Generation of an Electrochemical Gradient

Production of a semipermeable enclosed system enables the generation of an electrochemical gradient as a source of metabolic energy and this potential energy can be utilized by permitting the gradient to equilibrate by passing through a system which is coupled to an appropriate synthetic reaction. The mechanism of generating an electrochemical gradient involves electron transport across the containing membrane from an electron donor to an electron acceptor on the other side. Primitive systems may have used mechanisms based on external donors of electrons such as ammonia, sulphur dioxide, reduced iron and nitrite.

The basic system of generating an electrochemical gradient (of which an example still exists in Escherichia coli) requires linked exterior and interior catalytic reactions. In this case formate is oxidised to carbon dioxide and two electrons are transferred through an intermediate shuttle molecule to another catalytic site where fumarate is reduced to succinate. This latter reaction requires the donation of two protons and thus the electron transfer is the equivalent of a proton pump moving, in effect, one proton from the interior to the exterior of the organism. The electrochemical gradient achieved by this reaction is then able to drive important synthetic reactions such as ATP synthesis.

Autotropism: Evolution of Photochemical systems

The major evolutionary breakthrough was the development of autotropism in organisms by the evolution of photochemical reaction centres. This took place about 3×10^9 years ago in ancestors of the (present day) green sulphur bacteria. These prescient organisms took advantage of the photochemical principle enunciated by Einstein that a single quantum of light is sufficient to drive a chemical reaction. In the most primitive system (known as photosystem I) a magnesium-based porphyrin) absorbs one photon of light and excites an electron from the metal which is rapidly transferred to adjacent molecules in the complex producing a charge separation. The electron is passed to a shuttle mechanism, in this case ferredoxin, which passes it to an NADP reductase. The missing electron in the photoreceptor pigment is replaced by an electron from a suitable environmental donor molecule, in this case probably hydrogen sulphide since this has greater reducing potential (-230 mV compared to + 180 mV for water).

The next important step in the development of early living systems was the evolution of photosystem II in precursors of purple bacteria. This system uses light energy to excite electrons which are passed through a cytochrome complex which is able to use part of the energy to pump protons. The lower energy electron re-enters the cytochrome complex and is re-excited by light and passes round the system again. This is, perhaps, the first example of a purposeful electrical circuit.

Finally, a combination of both these photosystems in relatives of the cyanobacteria which occurred about 2.7×10^9 years ago gave rise to a system absorbing light energy which was able to use water (which was in plentiful supply) as the electron donor and which generated oxygen as the product. In this system the electron excited by the first quantum of light is used to drive a proton pump but is then passed to another

photosystem where absorption of a second quantum raises the redox potential of the electron which is used to reduce NADP. Various types of shunts and recycling are possible, for example the second photosystem can alternatively drive the proton pump mechanism.

Transition from Reducing to Oxidizing atmosphere

Clearly the great significance of the evolution of photosystems was the generation of oxygen. There appears to have been a delay in the rise of atmospheric oxygen. The large banded deposits of iron oxide dated about 2×10^9 years ago suggest that this lag in the rise of oxygen concentration was due to the oxidation of a large pool of reduced iron in the oceans. The rise in oxygen in the atmosphere is dated about 1.5×10^9 years ago and the advent of atmospheric oxygen changed the chemistry of living systems. As Szent-Gyorgyi has put it, "life processes changed from the α to the β state in which oxygen is the universal electron acceptor in the biosphere".

Evolution of microorganisms with oxygen-dependent metabolism

The abundance of organic molecules and oxygen permitted the evolution of microorganisms with electron transport systems adapted to transport electrons from NADH to oxygen. Such electron transport systems accept from NADH electrons which pass to oxygen through a series of proton-pumping complexes so that there is a gain in the electrochemical gradient. These systems were the forerunners of present-day mitochondrial electron transport.

Evolution of Eucaryotes (Evolution of Organelles/Endosymbiosis)

The next step in evolution seems to have involved phagocytosis of these organisms to form various types of endosymbiont. Probably the first step in this series involved autophagocytosis by a non-photosynthetic organism to give rise to the forerunners of nucleus and endoplasmic reticulum. Such a process would have the advantage of segregating the genetic material and increasing the effective surface area of the organism. It is possible that similar autoingestion processes gave rise to the Golgi apparatus and to peroxisomes.

Phagocytosis of oxygen-utilizing micro-organisms by primitive cells are thought to account for mitochondria, and plants may have evolved later by phagocytosis of photosynthetic bacteria to give chloroplasts.

All these organelles are self-replicating and contain genetic and/or epigenetic information required for their replication. Interestingly, genes coding for most of the components of mitochondria and chloroplasts have been transferred from these organelles to the nucleus. The reason for this is not clear but it is possible that this reduces the potential for oxidative damage to DNA at the sites of oxygen metabolism. Transfer into the nucleus may therefore preserve the integrity of otherwise vulnerable regions of the DNA of organelles. The consequence of the gene transfer is that these endosymbiotic organelles require either all or most of their components such as lipids and proteins to be synthesized elsewhere in the cell and be transferred to them. Delivery depends on phospholipid exchange proteins for phospholipids and on various complex sorting signals for proteins.

Protein traffic: Sorting Signals

The general scheme for protein traffic in the cell may be represented as a binary pathway; certain proteins remaining in the cytosol with some being transferred secondarily to the nucleus, mitochondria, chloroplasts or peroxisomes; and other proteins being translocated into the endoplasmic reticulum space (which is topologically exterior to the cell) and then variously transported to the Golgi apparatus and sorted into lysosomes, secretory vesicles and plasma membrane-destined vesicles. The signalling systems are extremely complex and the full process, involving a number of accessory proteins that assist translocation and sorting such as chaperonins, may be divided to three categories: (1) amino acid sequences in the form of signal peptides or signal "patches" in the folded protein; (2) modification of amino acids to generate secondary signals, such as phosphorylation of serine, methylation of glutamic acid, acetylation of lysine, the acylation of tyrosine, or the formation of acylated thiol esters at the carboxy terminus; (3) protein glycosylation, the major series being through the N-glycosylation of serine. Single sugar additions, such as in the N-acetylglucosamine "anchor" which attaches proteins to the cytosolic portion of the ER, or phosphatidyl inositol attachment to the carboxy terminus which seems to anchor some proteins to the inner surface of the endoplasmic reticulum, are relatively rare. The major process involves the dolichol-mediated oligosaccharide transfer which occurs on the interior of the endoplasmic reticulum.

Subsequent processing occurs by limited proteolysis, and modification of the oligosaccharides. These modifications include phosphorylation of mannose and reorganization of the components of the oligosaccharides and take place mainly in the Golgi apparatus. Transfer between the various compartments occurs by vesicular transport which carries proteins through the cis, medial and trans compartments of the Golgi apparatus where various signal modifications take place. The proteins are finally sorted into categories in the trans-Golgi network and give rise to the sets of vesicles currently recognized, i.e. lysosomes, secretory vesicles and plasma membrane vesicles. The mechanisms of budding of these vesicles appears to differ. Lysosomes appear to be formed from clathrin-coated vesicles containing mannose phosphate receptors. The sorting signals and budding mechanisms of other vesicular traffic are not clear.

The Nature of the Melanosome

With these processes of generation of intracellular organelles in mind we may begin to formulate a view about the nature of the organelle which is of specific interest to us as Pigment Cell Biologists - namely the melanosome. Although the long controversy in the past regarding the possible mitochondrial origin of melanosomes was effectively settled by the classic demonstration in Oxford by Seiji, Fitzpatrick and Birbeck (using the then novel technique of differential gradient centrifugation) that the melanosome could legitimately be regarded as a separate organelle, much of the present knowledge of melanosomes is the result of work carried out in Japan. As well as paying tribute to the work of Seiji we must acknowledge the important contributions of Jimbow and in particular the series of elegant studies by Mishima and his collaborators.

There has recently been a proposal by Gisella Moellman that the melanosome is a type of peroxisome. This suggestion was made on the basis of the catalase activity detected in melanosomes, and the amino acid sequence of tyrosinase which appears to

have three amino acids of the carboxy terminus which resemble the peroxisomal signal sequence. This is an interesting idea and is consistent with the historical suggestion of Medawar that pigment spread in vertebrates occurs by transfection of adjacent pigment cells by self-replicating particles.

However, there are a number of features which, in my view, are inconsistent with the peroxisomal hypothesis. For example, melanosomes have a single membrane structure and appear to grow by vesicular fusion, like endolysosomes. No evidence has been reported of replicating melanosomes. They do not contain nucleic acid. Therefore, if they were to be epigenetically transmitted like peroxisomes, melanosomes would need to be present in all (or most) cells, and certainly in oocytes. I am not aware of any evidence of such epigenetic transfer of melanosomes in most species (although there may be some examples e.g. amphibia).

Secondly, the tyrosinases that have been analysed are known to be glycosylated and therefore cannot be destined for cytosolic distribution and subsequent incorporation into a self-replicating organelle such as a peroxisome. Moreover, activity attributed to tyrosinase has been detected by many investigators in the trans-membrane domain.

Thirdly, co-segregation of tyrosinase activity appears to be (predominantly) with lysosomal enzymes. The enzymes that have been detected in isolated and purified melanosomes include: acid phosphatase, aryl sulphatase, beta-galactosidase, β -N-acetylglucosaminidase, beta-glucuronidase, cathepsin D and alpha-mannosidase. This points strongly to co-segregation of tyrosinase with lysosomal enzymes, although some other enzymes have also been detected such as tryptophan-2,3-dioxygenase, tyrosine aminotransferase, ATPase, and gamma-glutamyl transferase which are not classical lysosomal enzymes.

Fourthly, there is evidence from some recent work by Alison Winder that fibroblasts transfected with the tyrosinase gene generate pigment products in lysosome-like vesicles.

If we add to this the suggestion that the carboxyl terminus of the tyrosinase is in the transmembrane portion of the enzyme (and therefore could not act as a signal for transfer to peroxisomes) and the recent demonstration by Sandra Naish-Byfield that the catalytic activity of tyrosinase is, at least in part, due to alternative copper-centred catalysis, I believe the case for suggesting that melanosomes are peroxisome-like organelles is greatly weakened.

If the melanosome is not a peroxisome in what category does it belong? From an evolutionary point of view it is probably a secretory vesicle since for example, in arthropods tyrosinase is externalised for its role in cuticular hardening. Also, in a sense, the triggered release of products such as in the spray glands of millipedes and bombardier beetles and certain cephalopods suggests a secretory role for the organelle. However, in higher plants and animals the vesicle is generally retained in the cell - although not invariably.

Evolution of Tyrosinase (Trends in Phylogeny)

In the presence of so distinguished and knowledgeable an audience, it may be an error to venture into this dark forest of speculation. However, as this is a privileged occasion, I will touch on the subject. It would appear that the ancestral gene occurred very early in evolution, since tyrosinase appears to be a feature both of plants and animals. In the animal kingdom some divergence seems to have taken place leading to haemocyanins in the descendants of the protostomes.

Since evolution is conservative what could have given rise to tyrosinase? One possibility, I venture to suggest, might be from a precursor gene for cytochrome a since cytochrome a and a₃ have a copper-binding site and also haem attachment sites. It is possible that such an origin could account for some of the features of the aminoacid sequence that are of current interest.

Evolutionary Significance of Orthoquinones

The major significance of tyrosinase appears to be as a mechanism for generating reactive orthoquinones. Orthoquinones have a different electronic structure, and different reactivity, to paraquinones. The latter are much used by living systems in electron transport as reversible electron acceptors and donors. In general, orthoquinones more readily undergo covalent binding to the ring by nucleophiles. Their evolutionary function may be connected with this property and orthoquinones appear to be important in micro-organisms, including fungi and bacteria as a type of generalised antibiotic. Orthoquinones also appear to be significant in protecting plants, and particularly fruits, from opportunistic predators such as insects or grubs, and we are all familiar with the blackening of fruits (e.g. bananas) when they are damaged. Orthoquinones are used by insects in their immune system and also in defensive sprays. In all probability they also form a significant repellent component of cephalopod ink. Another protective function appears to be the use of orthoquinones in strengthening protein coats by tanning of spores and seed pods and, of course, in the sclerotization of the insect cuticle.

Naturally, the chemical reactivity of orthoquinones also means that they are potentially precursors of polymeric pigments which we term melanin. It seems not improbable that melanogenesis evolved as a detoxification pathway for orthoquinones generated for the purposes to which I have just alluded. Polymerisation may have been utilized to maintain an acceptable steady-state concentration of orthoquinone either at the periphery of the organism or in melanosomes.

Whilst the precise mechanism of melanogenesis has not yet been clarified, the process finally results in the generation of a bathochromic indolic polymer of irregular structure with important physicochemical properties, including a wide spectral absorption, semiconductor properties, stable radical properties, phonon-photon coupling, easy formation of charge-transfer complexes, and strong cationic binding. The biological applications and significance of these properties is properly the topic of another dissertation.

Conclusion

I am conscious that what I have presented is a sketchy and speculative evolutionary view of melanogenesis; it has, of course, posed more questions than it has answered.

Nevertheless, I believe that our salvation as a species lies in our rationality and we are bound together by having Science as our philosophy (I mean 'Science' in the Popperian sense). In this discourse I have employed the principle of parsimony, first expounded by William of Ockham but, of course, we must remain cognizant of the complexity of our universe. You will have detected that I am guided by the maxim of Alfred North Whitehead 'seek simplicity and distrust it'.

Acknowledgements

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I am grateful to Dr Wiete Westerhof, Chairman of the Organizing Committee for the invitation to give this lecture. I am indebted to many individuals who have helped, directly or indirectly, to imbue me with knowledge and to mould my views. In particular I thank Dr Jan Borovansky, Professor Peter Campbell, Dr Sandra Naish-Byfield and Dr Anthony Smith. Any blame, however, attaches to me alone.

CURRENT LITERATURE

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

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An NMR study of the interaction between melanin free acid and Mn²⁺ ions as a model to mimic the enhanced proton relaxation rates in melanotic melanoma. Magn Reson Imaging 9:963-968, 1991.
Abstract : The interaction of a soluble Melanin Free Acid (MFA) from Sepia melanin with Mn²⁺ ions is investigated by measuring the proton water relaxation rates. The similarity between MFA and the parent melanin is assessed by means of their high resolution ¹³C cross polarization magic angle spinning NMR spectra. The observed marked increase in longitudinal proton relaxation rates and the characteristic 1/T₁ NMRD profile are associated to the formation of a macromolecular metal complex. The presence of similar paramagnetic species is expected to cause the high contrast shown by melanotic tissues in MRI.
- Ben-Shachar D, Riederer P, Youdim MB.
Iron-melanin interaction and lipid peroxidation: implications for Parkinson's disease. J Neurochem 57:1609-1614, 1991.
Abstract : The vulnerability of substantia nigral (SN) melanized dopamine neurons to neurodegeneration in Parkinson's disease and the selective increases of iron and basal lipid peroxidation in SN indicate that iron-melanin interaction could be crucial to the pathogenesis of this disease. The present study describes, for the first time, the identification and characterization of a high-affinity (K_D = 13 nM) and a lower affinity (K_D = 200 nM) binding site for iron on dopamine melanin. The binding of iron to melanin is dependent on pH and the concentration of melanin. Iron chelators, U74500A, desferrioxamine, and to less extent 1,10-phenanthroline and chlorpromazine, but not the Parkinson-inducing neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, can inhibit the binding of iron to melanin and iron-induced lipid peroxidation. Although melanin alone diminishes basal lipid peroxidation in rat cortical homogenates, it can also potentiate that initiated by iron, a reaction inhibited by desferrioxamine. In the absence of an identifiable exogenous or endogenous neurotoxin in idiopathic Parkinson's disease, iron-melanin interaction in pars compacta of SN may be a strong candidate for the cytotoxic component of oxygen radical-induced neurodegeneration of melanized dopamine neurons.
- Costa C, Bertazzo A, Allegri G, Catinella S, Traldi P.
Hydroxyindole intermediates in the process of melanogenesis: a mass spectrometric study. J Heterocycl Chem 28:2021-2024, 1991.
Abstract : Studies on the enzymic formation of polymers from hydroxyindoles are described. Fast atom bombardment (FAB) anal. of the reaction mixts. followed by collisional spectroscopy of the most abundant ionic species was applied to analyze products obtained from the reaction catalyzed by tyrosinase with 4- or 5-hydroxyindole at different times. The results suggested that moieties of the precursors are still present in the oligomeric systems. Among the reaction products, dimeric and trimeric compds. were detected by FAB and their structure assignment was achieved by collision spectroscopy.

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Oxidative coupling of DOPA with resorcinol and phloroglucinol: isolation of adducts with an unusual tetrahydromethanobenzofuro[2,3-d]azocine skeleton. Tetrahedron 47:6243-6250, 1991.
Abstract : Under biomimetic conditions, both phloroglucinol and resorcinol cause significant inhibition of the oxidative conversion of dopa to dopachrome, a key step in the biosynthesis of melanin pigments. The major product ($\lambda_{max} = 377 \text{ nm}$) from oxidn. of dopa in the presence of phloroglucinol was methanobenzofuroazocine I (R = OH, R1 = H). Another product, formed in a smaller amt., was diadduct II which arose from coupling of phloroglucinol with 2 dopaquinone derived units. Analogous cross coupling adducts I (R = R1 = H; R = H, R1 = Me) were obtained by reaction of dopa or dopa methylester with resorcinol, resp. These results suggest the trapping of labile dopaquinone, generated in situ from oxidn. of dopa, by the reactive phenolic compds., leading to the novel adducts I (R = OH, R1 = H; R = R1 = H; R = H, R1 = Me).

- Duff GA.
Carbon-13 nuclear magnetic resonance spectroscopy of isotopically substituted porphyrins adsorbed to melanins. Diss Abstr Int B 52:802, 1991.

- Pawelek JM.
After dopachrome? Pigment Cell Res 4:53-62, 1991.
Abstract : Dopachrome, an intermediate in melanin biosynthesis, exhibits some unusual properties. At physiologic pH (e.g., pH 6-8) it is unstable and spontaneously loses its carboxyl group to form 5,6-dihydroxyindole (DHI) and CO₂. However, over this same pH range, if various metals or melanocyte-specific enzyme are present, it rapidly rearranges to its isomer form --5,6-dihydroxyindole-2-carboxylic acid (DHICA)-- which is far more stable than dopachrome in its ability to retain the carboxyl group. Whether or not the carboxyl group is retained could have important implications for the regulation of melanogenesis, since in the presence of oxygen DHI spontaneously forms a black precipitate, whereas DHICA forms a golden-brown solution. The solubility of "DHICA-melanin" is due to the presence of carboxyl groups, which provide negative charges and hydrophilicity. Thus, in vivo, the extent to which dopachrome is converted to DHI or DHICA may well influence the solubility and color of the melanin formed. The purpose of this article is to review recent findings in these areas and to discuss the possible significance of dopachrome conversion in the regulation of melanogenesis and color formation.

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In vitro scavenger activity of some flavonoids and melanins against O₂⁻(.). Free Radic Biol Med 11:1-8, 1991.
Abstract : The scavenger activity against O₂⁻. of some flavonoids and melanins (synthetic melanins and melanins isolated from animal tissues, vegetable seeds, and mushroom spores) has been studied by ESR spectrometry. All these substances, except flavon and flavanone, diminish the signal of O₂⁻. generated in vitro by a system containing H₂O₂ and acetone in an alkaline medium. It is shown that the presence of hydroxyl groups in the B ring of flavonoids is essential for their scavenger activity. Moreover, the presence of a hydroxyl at C-3 enhances the scavenger ability of flavonoids. Generally, aglycons are more active than their glycosides. It seems plausible that the antioxidant property of these substances comes from their scavenger activity against O₂⁻(.). It is also pointed out that the scavenger activity shown by melanins, is strictly correlated with their nature of stable free radical.

- Singh S, Dryhurst G.
Interactions between 5,6-dihydroxytryptamine and cysteine. Bioorg Chem 19:274-282, 1991.
Abstract : Autoxidn. and enzyme-mediated oxidns. of the serotonergic neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) generate the corresponding ortho-quinone (I) as the initial reactive intermediate. Autoxidn. of 5,6-DHT in the presence of an excess of the cellular reductant/nucleophile cysteine results in a reaction where, initially, I oxidizes cysteine to cystine with concomitant formation of 5,6-DHT. When the concn. of cysteine is sufficiently reduced I attacks, 5,6-DHT to give 2,7'-bis(5,6-dihydroxytryptamine) and, ultimately, an insol. indolic melanin. When the oxidn. of 5,6-DHT is catalyzed by tyrosinase, I is attacked by cysteine to give 4-S-cysteinyl-5,6-

dihydroxytryptamine (II). Methods have been devised to isolate II and evidence for its structure is presented. Conjugate II should be useful as a marker of enzyme-catalyzed oxidn. of 5,6-DHT in the central nervous system.

- Vsevolodov EB, Ito S, Wakamatsu K, Kuchina II, Larypov IF.
Comparative analysis of hair melanins by chemical and electron spin resonance methods. Pigment Cell Res 4:30-34, 1991.

Abstract : Eighteen hair samples from Karakul newborn lambs with various colors were estimated for eumelanin and pheomelanin contents (Ce and Cp, respectively) by electron spin resonance (ESR) spectrometry and by high-performance liquid chromatography (HPLC). Correlation coefficients between the values estimated by the ESR and HPLC methods were 0.96, 0.93, and 0.99 for Ce, Cp, and Ce/Cp, respectively. The high correlation coefficients show that both methods fit well for estimation of relative values of these parameters. The absolute values of Ce and Ce/Cp coincide rather well when Ce is high, but considerable discrepancies appear when Ce is low. The reasons for these discrepancies are discussed. The HPLC method appears to be more sensitive for detection of low concentrations of pheomelanin, while the ESR method fits well for mass selection purposes.

2. Biology of pigment cells and pigmentary disorders

- Agin PP, Dowdy JC, Costlow ME.
Diacylglycerol-induced melanogenesis in Skh-2 pigmented hairless mice. Photodermatol Photoimmunol Photomed 8:51-56, 1991.

Abstract : We investigated the effect of topically applied diacylglycerols (DG) on melanogenesis in Skh-2 pigmented hairless mouse skin. Groups of mice were treated according to 4 different regimens of either 1,2-dioctanoyl-sn-glycerol (DOG) or 1-oleyl-2-acetyl-sn-glycerol (OAG) with or without ultraviolet irradiation (UVR). After the treatment regimens were completed, separated epidermal tissue was stained with L-dopa and thin sections of whole skin were stained by the Warthin-Starry method to detect melanin deposition. Quantification of the stained areas by digital image analysis disclosed that DOG treatment without UVR increased the dopa-positive area in skin in a dose-dependent manner but had no effect on melanin deposition. DG treatment acted synergistically with UVR to enhance melanogenesis, with synergism being more pronounced for melanin deposition than for dopa staining. DOG treatment prior to UVR also resulted in an enhanced melanogenic response to UVR, suggesting that DG increases the sensitivity of melanocytes to subsequent UVR by inducing dopa oxidase activity. OAG also enhanced UVR-induced melanogenesis in a dose-dependent manner and was at least as potent an inducer as was DOG. Because DG is known to activate protein kinase C, our results suggest that a protein kinase C-dependent process is involved in melanogenesis.

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Inhibitory effect of arbutin on melanogenesis--biochemical study using cultured B16 melanoma cells. Nippon Hifuka Gakkai Zasshi 101:609-613, 1991.

Abstract : Inhibitory effect of arbutin (hydroquinone-beta-D-glucopyranoside) on the melanogenesis was studied biochemically using cultured B16 melanoma cells. The maximum arbutin concentration lacking an inhibitory effect on cell growth was 5×10^{-5} M. At this concentration, melanin content per cell was decreased significantly to about 39%, compared with that of arbutin untreated cells. Also, tyrosinase activity of arbutin treated cells was decreased significantly. When arbutin was added to B16 melanoma cell suspension, arbutin was not hydrolyzed to liberate hydroquinone. Further, tyrosinase activity in crude preparations from B16 melanoma cells was inhibited by arbutin. From these results, it is suggested that arbutin can inhibit the melanogenesis by affecting not only the synthesis but also the activity of tyrosinase rather than by killing melanocytes B16 melanoma cells. Also, it is suggested that hydroquinone is not responsible for the inhibitory effect of arbutin on the melanogenesis.

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EPR spectroscopic determination of pigment type in the wool of fine-fleeced and semi-fine-fleeced

brown sheep. Dokl Vses Akad S-kh Nauk im V.I. Lenina 5:38-40, 1991.

Abstract : EPR spectroscopy was used to show the absence of the pheomelanin component in melanosomes of brown wool of Australian Merino and Romney Marsh sheep. The genotype of these sheep was Aa/AaBb/Bb, different from reddish-brown Asiatic sheep controlled by interactions of the AWt gene with alleles EBl, EBr, RY.

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Characterization of a naturally occurring protease inhibitor in the hemolymph of the scorpion, *Heterometrus bengalensis*. Dev Comp Immunol 15:213-218, 1991.

Abstract : A protease inhibitor has been purified by ultracentrifugation, affinity chromatog. on trypsin-sepharose 4B, and chromatofocusing on PBE-94 from hemolymph of the scorpion *H. bengalensis*. Homogeneity of the protease inhibitor was demonstrated by HPLC. The protease inhibitor is a monomeric glycoprotein with a mol. wt. of 120,000 daltons, which is stable between pH 4 and pH 8. The mol. inhibits serine proteases like trypsin and .alpha.-chymotrypsin and shows a noncompetitive mode of inhibition towards trypsin, with a Ki of 6.1 .times. 10⁻⁶ mM. Amino acid anal. shows a preponderance of aspartic acid, glutamic acid, serine, and glycine. The protease inhibitor is efficient in inhibiting phenoloxidase activity in both the hemolymph and the isolated phenoloxidase. Melanin synthesis by phenoloxidase may be influenced by this protease inhibitor.

- Barrett AW, Beynon AD.

A histochemical study on the distribution of melanin in human oral epithelium at six regional sites. Arch Oral Biol 36:771-774, 1991.

Abstract : Oral mucosa from six sites in 95 autopsies was tested for melanin using the Masson-Fontana silver reduction method. Melanin was detected in 51.6% of labial, 46.3% of palatal, 45.3% of buccal, 28.4% of mandibular gingival, 25.3% of lingual and 21.1% of maxillary gingival samples. 93.7% of epidermal samples from the same population were positive. In 24.2% of the subjects there was no detectable melanin at any intraoral site and 4.2% showed activity in all six sites. The mean number of positive oral sites per individual was 2.2. There are thus regional differences in oral epithelial melanocyte activity, but no parallel with the known regional incidence of primary oral melanoma.

- Bartosik J.

Melanosome complexes and melanin macroglobules in normal human skin. Acta Derm Venereol (Stockh) 71:283-286, 1991.

Abstract : The presence of melanin macroglobules, and sometimes that of melanosome complexes also, in epidermal melanocytes has been considered a feature of various skin diseases. Opinions differ as to whether these structures can occur in normal skin. We have studied these melanin inclusions in normal Caucasian skin in the entire soma of 116 melanocytes and the occurrence of melanosomes in phagosomes of 77 Langerhans' cells obtained in different seasons. During winter the melanocytes contained few melanosomes but many melanosome complexes and melanin macroglobules. These melanosome inclusions were in 86%, localized in the most basal part of the melanocytes, particularly in the dermal protrusions. It is suggested that these structures can be transferred from epidermal melanocytes to dermal cells and that melanin macroglobules derive from melanosome complexes. Irrespective of the season, most of the Langerhans' cells contained melanosomes in their phagosomes, which suggests a phagocytic capacity of these cells and a role in the elimination of the melanin.

- Borovansky J, Mirejovsky P, Riley PA.

Possible relationship between abnormal melanosome structure and cytotoxic phenomena in malignant melanoma. Neoplasma 38:393-400, 1991.

Abstract : Melanogenesis has been regarded as a hazard for pigment cells which are endangered by reactive quinones and semiquinones generated by this process. Normally the potentially cytotoxic species are confined to melanosomes by a limiting membrane and thus separated from the rest of the cell. Our electron microscopic investigation has demonstrated the presence of abnormal and incomplete melanosomes in human melanomas from epidermal and mucosal sites, in melanoma metastases, and in B16 mouse melanoma. We conclude that significant leakage of reactive melanin

precursors including free radical species may occur from aberrant melanosomes in pigmented tumors. This would be expected to be reflected by fully extended physiological scavenging mechanisms, and by local and distant manifestations of cytotoxicity: Among these manifestations is free radical damage to the liver, detected by a thiobarbituric acid-reactive substances assay, in B16 melanoma-bearing mice. The efflux of toxic species from abnormal melanosomes may explain both the observed frequent occurrence of necrosis in melanomas and the therapeutic efficacy of tyrosinase substrates and may also be one of the factors influencing the extent of melanogenuria.

- Cable J, Steel KP.

Identification of two types of melanocyte within the stria vascularis of the mouse inner ear. *Pigment Cell Res* 4:87-101, 1991.

Abstract : We have distinguished two types of melanocyte within the intermediate layer of the stria vascularis in the cochlea of normally pigmented mice: light and dark intermediate cells. The light intermediate cells are present in the stria from birth and have the typical appearance of a melanocyte. They are large and dendritic with electron-lucent cytoplasm containing numerous vesicles that show tyrosinase activity, and pigment granules in various stages of development. These granules have the ultrastructural and histochemical characteristics of premelanosomes and melanosomes. The light intermediate cells persist throughout life, but less frequently contain pigment in older animals. The dark intermediate cells, present only in adult mice, vary considerably in number and distribution between animals. Pigment granules, bound within an electron-dense acid phosphatase-rich matrix, form the main component of the dark intermediate cells. The intermediate cells may comprise either two distinct cell populations or different developmental stages of the same cell type; ultrastructural observations suggest the latter. In young mice, light intermediate cells contain the electron-dense matrices, which at later stages of development are found almost exclusively in dark cells. The dark intermediate cells contain few cell organelles other than pigment granules accumulated within lysosomal bodies and they often have pycnotic nuclei. These observations suggest that the dark intermediate cells are a degenerate form of the light intermediate cells. Clusters of melanosomes also occur in the basal cells, and to a much lesser extent in the marginal cells. These cells do not stain after incubation in DOPA, suggesting that they are not capable of melanin synthesis, and therefore probably acquire melanin by donation from adjacent melanocytes. Pigment clusters are also found within the spiral ligament at all stages of development.

- Chirila TV, Cooper RL, Constable IJ, Richardson GW, Vijayasekaran S.

"Black prosthesis" revisited: a study of epinephrine-induced pigment deposits on poly(methyl methacrylate). *Graefes Arch Clin Exp Ophthalmol* 29:578-582, 1991.

Abstract : To investigate in detail the "black prosthesis" syndrome, experimental production of melanin from epinephrine was performed both in bulk and onto the surface of a common prosthetic material, poly(methyl methacrylate) (PMMA). The study by ultraviolet/visible spectrometry showed that the radiation-absorptive properties of PMMA were significantly enhanced; a sample treated for 20 days in epinephrine absorbed all ultraviolet radiation up to a 344-nm wavelength and transmitted only 4.9% from the ultraviolet spectrum at 400 nm and 16.2% from the visible spectrum at 500 nm. Transmission electron microscopy studies suggest that melanogenesis occurs on the surface of PMMA, and the pigment does not penetrate the polymer. Using infrared spectrometry, it was confirmed that the pigmentation is caused by a melanin formed through the oxidative polymerization of epinephrine.

- Gudz SP, Kostruba MF, Girna OV.

Melanin formation and tyrosinase activity in *Flavobacterium leopoliensis*. *Mikrobiol Zh (Kiev)* 52:21-23, 1990.

Abstract : Cells of *Flavobacterium leopoliensis* synthesized melanin only on media contg. tyrosine. A dependence between pigment formation and tyrosinase activity of the cells was found. Melaninogenesis was considerably stimulated by intensive aeration, Cu, nitrates, and peptone.

- Haneke E.

Laugier-Hunziker-Baran syndrome. *Hautarzt* 42:512-515, 1991.

Abstract : Laugier-Hunziker-Baran syndrome is characterized by lenticular melanin pigmentation of

the lips and oral mucosa that may be associated with longitudinal melanonychia. Neither a systemic nor a local cause is demonstrable. There is no risk of development of oral or subungual malignant melanoma. Fifteen cases of this syndrome, which has not yet been reported in the German literature, are described.

- Hearing VJ, Tsukamoto K.

Enzymatic control of pigmentation in mammals. FASEB J 5:2902-2909, 1991.

Abstract : Visible pigmentation in mammals results from the synthesis and distribution of melanin in the skin, hair bulbs, and eyes. The melanins are produced in melanocytes and can be of two basic types: eumelanins, which are brown or black, and pheomelanins, which are red or yellow. In mammals typically there are mixtures of both types. The most essential enzyme in this melanin biosynthetic pathway is tyrosinase and it is the only enzyme absolutely required for melanin production. However, recent studies have shown that mammalian melanogenesis is not regulated solely by tyrosinase at the enzymatic level, and have identified additional melanogenic factors that can modulate pigmentation in either a positive or negative fashion. In addition, other pigment-specific genes that are related to tyrosinase have been cloned which encode proteins that apparently work together at the catalytic level to specify the quantity and quality of the melanins synthesized. Future research should provide a greater understanding of the enzymatic interactions, processing, and tissue specificity that are important to pigmentation in mammals.

- Herraiez MP, Zapata AG.

Structural characterization of the melano-macrophage centres (MMC) of goldfish *Carassius auratus*.

Eur J Morphol 29:89-102, 1991.

Abstract : In the present work we have studied the organization of melano-macrophage centres (MMCs) in the peripheral lymphoid organs, including spleen, pro- and mesonephros, of the goldfish, *Carassius auratus*, in an attempt to clarify their cellular composition, origins and functional relationships. Histological analysis demonstrated a similar organization in the three organs on the basis of closely packed phagocytic cells containing abundant pigment. The MMCs of *Carassius auratus* are found throughout the parenchyma of spleen and kidney and show a close association with the vascular system, i.e. splenic ellipsoids, sinusoids of red pulp and renal blood sinuses. They exhibit distinct degree of development from small groups of actively phagocytic macrophages to large, totally or partially encapsulated centres, where effete phagocytic cells are filled by cell debris. Ultrastructural and histochemical data suggest that the main inclusion observed in the MMCs of *Carassius auratus* is lipofuscin. Haemosiderin occurs in lesser amounts and melanin is almost restricted to kidney MMCs,--mainly mesonephros--. Our results suggest various non-specific physiological roles for the teleost MMCs, including tissue breakdown and erythrocyte catabolism.

- Hill HZ, Cathcart KN, Bargellini J, Trizna Z, Hill GJ, Schallreuter KU, Wood JM.

Does melanin affect the low LET radiation response of Cloudman S91 mouse melanoma cell lines? Pigment Cell Res 4:80-86, 1991.

Abstract : Melanin contains melanin-free radicals and can both absorb and produce additional free radicals and active oxygen species on exposure to various stimuli. Yet its role in the radiation responses of malignant melanoma has been little studied. In this report, three subclones of Cloudman S91 mouse melanoma clone PC1A varying in constitutive melanin content were compared with respect to killing by gamma irradiation. Radiation responses correlated with melanin content. The least melanotic line, S91/amel, was most sensitive and the most melanotic line, S91/I3, was most resistant. Curve fitting using the linear-quadratic model suggests that S91/amel is killed only by single event inactivations; S91/I3, only by double event inactivations; and S91/M1B, with intermediate melanin and radiation response, by both types of inactivations. Split dose experiments confirmed a lack of immediate split dose recovery in S91/amel and its existence in S91/I3. Potentially lethal damage and its repair could be demonstrated in both S91/amel and S91/I3. Double strand break (DSB) induction was evaluated as a function of gamma ray dose in DNA of S91/I3 and S91/amel, as well as in EMT6, a mouse mammary cancer line that lacks tyrosinase and melanin. The rates of induction were proportional to cellular melanization, i.e., the rate of DSB induction was greatest in S91/I3, least in EMT6. Levels of thioredoxin reductase (TR), glutathione reductase (GR),

superoxide dismutase (SOD), and catalase (CAT) were determined in S91/amel and S91/13. TR was the same in both cell lines, while the other three enzymes were 3- to 4-fold lower in S91/amel.

- Hoeffding V, Fechter LD.

Unusual morphology of the stria vascularis in pigmented strain 2/NCR guinea pigs. *Hear Res* 54:39-44, 1991.

Abstract : This paper reports an abnormality in the morphology of the apical stria vascularis of inbred 2/NCR guinea pigs as compared to outbred animals. Cochleas were embedded in plastic, sectioned, and examined in the light and electron microscopes. In the 2/NCR animals, the apical stria vascularis consisted of a cuboidal epithelium composed of a monolayer of poorly differentiated cells. Few or no capillaries were associated with this epithelium. No melanin pigment was present in the abnormal region of the stria in these animals, although pigmentation appeared normal in lower turns of the cochlea. Measurements of compound action potential thresholds between 2 and 40 kHz revealed no differences in auditory function between the two strains.

- Jimbow K.

N-acetyl-4-S-cysteaminyphenol as a new type of depigmenting agent for the melanoderma of patients with melasma. *Arch Dermatol* 127:1528-1534, 1991.

Abstract : Melasma is a difficult medical problem to treat. Hydroquinone is administered to many patients, but it is unstable and local irritation and dermatitis may develop after a prolonged use at a high concentration. This study introduces a new depigmenting agent, N-acetyl-4-S-cysteaminyphenol, for better management of melanoderma in patients with melasma. Our study, based on a retrospective observation of 12 patients using 4% N-acetyl-4-S-cysteaminyphenol in oil-in-water emulsion, showed a complete loss (8%), a marked improvement (66%), or a moderate improvement (25%) of melasma lesions. Visible changes of melanoderma can be seen in 2 to 4 weeks after daily topical application. This depigmentation was associated with a decrease in the number of functioning melanocytes and in the number of melanosomes transferred to keratinocytes. N-acetyl-4-S-cysteaminyphenol is the tyrosinase substrate, and, on exposure to tyrosinase, it formed a melanin-like pigment. A phenolic thioether, N-acetyl-4-S-cysteaminyphenol, is a new type of depigmenting agent for the better management of melasma. It is much more stable and less irritating to the skin than hydroquinone, and it is specific to melanin-synthesizing cells.

- Keyhani-Rofagha S, Kooner DS, Landas SK, Keyhani M.

Black thyroid: a pitfall for aspiration cytology. *Diagn Cytopathol* 7:640-643, 1991.

Abstract : Black thyroid discoloration following long-standing use of minocycline has been reported. Morphologic findings of aspiration cytology of these lesions was first reported from The Ohio State University. This abstract describes a second case of black thyroid that was preceded by aspiration cytology. In both cases, thyroidectomies were performed based on "indeterminate" fine-needle aspiration cytology (FNAC). Degenerative changes in follicular epithelial cells in black thyroid causes nuclear hyperchromasia and chromatin clumping, which may be mistaken for neoplasia. Pigment present in follicular epithelial cells and macrophages may be obscured by pigments with similar microscopic appearances, such as hemosiderin. A clinical history of the chronic use of a tetracycline derivative should alert the pathologist to the possibility of black thyroid. Diagnosis may be made by applying special stains on the cell block. The pigment stains with Melanin stain (Fontana) and bleaches with potassium permanganate. In the light of increasing use of FNAC of the thyroid gland and the large number of patients who received tetracycline therapy in the last two decades, it is likely that some practicing cytopathologists may experience this pitfall.

- La D.

Alcaptonuria and ochronotic arthritis. *Mol Biol Med* 8:31-38, 1991.

Abstract : The rare hereditary metabolic disorder alcaptonuria is characterized by the inability to metabolize homogentisic acid, an intermediary compound in the catabolism of the aromatic amino acids phenylalanine and tyrosine. The essentially complete deficiency of homogentisic acid oxidase causes a striking accumulation of homogentisic acid and a derived melanin-like pigment in the connective tissues; the latter is termed ochronosis. Urinary homogentisic acid is oxidized rapidly and

becomes a brown or black pigment if alkali is added. Older alcaptonurics have intensely pigmented (ochronotic) connective tissues, primarily the cartilaginous joint surfaces, ribs, intervertebral disks, ear cartilage, etc. They also have an unusual type of arthritis affecting the large weight-bearing joints, i.e. hips, knees and spine, but not the small joints of the hands and feet, as in rheumatoid arthritis. A mechanistic explanation for ochronotic arthritis has not been worked out, but it is clear that accumulation of homogentisic acid in the connective tissues directly or indirectly leads to the arthritic changes. A detailed analysis of the events leading to alcaptonuric arthritis should be worthwhile since it is a model form of arthritis secondary to a well-defined metabolic disorder that must persist for many years before the arthritic complications appear. Possibly other, more common types of arthritis, develop secondarily to metabolic disturbances that involve chemical mediators less obvious, or less easily detected, than homogentisic acid.

- Land EJ.
CRC-BACR-AICR International Workshop on Melanogenesis: its chemistry as a therapeutic strategy in melanoma. Br J Cancer 64:793-795, 1991.
- Land EJ.
AICR-BACR-CRC International Workshop on Melanogenesis: its chemistry as a therapeutic strategy in melanoma. Pigment Cell Res 4:63-65, 1991.
- Milman N.
Hereditary haemochromatosis in Denmark 1950-1985. Clinical, biochemical and histological features in 179 patients and 13 preclinical cases. Dan Med Bull 38:385-393, 1991.
Abstract : During the period 1950-1985, a total of 179 cases of clinically overt hereditary haemochromatosis (HH) were registered in Denmark, 140 males and 39 females. Median age at diagnosis was 55 years (range 29-81). Diagnostic approaches, symptoms and physical signs at discovery are described. All patients had grade 3-4 liver haemosiderin iron, and cirrhosis was present in 84%. Serum (S-) transaminase was elevated in 92%, S-alkaline phosphatase in 47% and S-bilirubin in 23%, while plasma prothrombin time was below normal in 34%. Females had higher alkaline phosphatase than males (p less than 0.05). Bone marrow haemosiderin iron (n = 81) showed no relation to iron status indicators and was unsuitable as a diagnostic tool. Skin biopsy (n = 56) was positive for haemosiderin iron in 67% and for melanin in 57%, but was of limited value in the assessment of HH. Arthropathy was registered in 44%; arthralgias and clinical joint abnormalities occurred more frequently in females than in males (p less than 0.05). Latent diabetes mellitus was found in 34% and overt diabetes in 55%, being more frequent in males than in females (p less than 0.05). Other endocrine abnormalities were seen in 66%. Cardiac failure was observed in 9% and abnormal ECG in 35%. Males had higher haemoglobin (p less than 0.0001) and S-iron (p less than 0.01) than females, while S-transferrin, transferrin saturation, S-ferritin and mobilizable iron stores showed no significant sex differences. Median transferrin saturation was 87% (range 52-100); values greater than 62% were observed in 96% of the patients. Median S-ferritin was 3,400 micrograms/l (800-12,700) and median iron stores 14.8 g (4.5-36.4).
- Montagna P, Procaccianti G, Galli G, Ripamonti L, Patrizi A, Baruzzi A.
Familial hypomelanosis of Ito. Eur Neurol 31:345-347, 1991.
Abstract : Two siblings presented the typical skin changes of hypomelanosis of Ito (HI) associated with mental and cerebellar signs. Their mother showed only the skin changes of HI but no neurological disturbances. HI is a hereditary disorder, in which familiarity may go unnoticed because of the different expressions of neural and cutaneous features.
- Ogawa T, Shinomiya T, Katsuoka K, Nishiyama S.
Comparative study on melanogenesis and cornification induced by three kinds of vitamin D3 in brown guinea-pig skin and on melanogenesis of cultured B16 melanoma cells. Nippon Hifuka Gakkai Zasshi 101:1109-1117, 1991.
Abstract : Application of 0.25.µg 1.alpha.,25-dihydroxyvitamin D3 (1.alpha.,25-VD) and 1.alpha.-hydroxyvitamin D3 (1.alpha.-VD) to back skin of brown-hair guinea pig reduced the Y value of skin

color by 5.9 and 3.8, resp. 1.alpha.,25-VD and 1.alpha.-VD reduced the Y values of hair color by 8.7 and 3.3, resp., and reduced hair growth by 86 and 93% of control, resp. The melanin content in hair increased to 130 and 115% by 1.alpha.,25-VD and 1.alpha.-VD, resp. Both compds. thickened the skin, and 1.alpha.,25-VD and 1.alpha.-VD increased DOPA-pos. cells by 2.5 and 1.7 fold, resp., and increased keratinocyte release from skin by 5.7 and 1.8 fold, resp. Vitamin D3 reduced the release to 0.7 fold. 1.alpha.,25-VD increased DOPA uptake by 113% in B16 cultured melanoma cells. No increase was detected by 1.alpha.-VD and vitamin D3. The compds. did not increase thymidine uptake.

- Pankovich JM, Jimbow K.

Tyrosine transport in a human melanoma cell line as a basis for selective transport of cytotoxic analogues. *Biochem J* 280:721-725, 1991.

Abstract : Tyrosine is an essential amino acid for the initial step of melanin synthesis, yet little is known concerning its transport in melanocytes. As an important first step in the development of new anti-melanoma agents based upon chemical and pharmacological modifications of melanin synthesis, the present study characterized the transport mechanism of tyrosine in vitro using the human melanoma cell line SK-MEL 23. Several tyrosine transport systems may be involved in melanocytes: systems L and T, which transport neutral amino acids with branched or aromatic side chains, and systems A and ASC, which transport neutral amino acids with smaller side chains. In order to determine which system or combination of systems is involved in tyrosine transport in melanoma cells, studies of kinetics, Na(+)-dependence and competitive inhibition were undertaken. The Km and Vmax. for the Na(+)-independent transport system were found to be 0.164 +/- 0.016 mM and 21.6 +/- 1.1 nmol/min per mg of protein respectively. This transport was preferentially inhibited by the system L specific analogue, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, the system T substrate tryptophan, and the sulphur homologue of tyrosine, 4-S-cysteinylphenol. Sequential addition of these inhibitors at increasing concentrations indicated that they inhibit the same transporter. Our results suggest that tyrosine transport in SK-MEL 23 melanoma cells is similar to system L transport previously characterized in other cell types. This one transport system appears to supply all the tyrosine required for both cell growth and melanin synthesis. The transport system may be subject to manipulation by melanogenic stimulating factors, making the transport of cytotoxic tyrosine analogues an important area for further study.

- Prasolova LA, Bazhan NM, Vsevolodov EB, Latypov IF.

Verification of the color genotype based on morphological analysis of fur pigmentation of brown and black water voles *Arvicola terrestris* L. *Genetika* 27:1423-1430, 1991.

Abstract : Qualitative and quantitative analysis of fur pigmentation in brown and black water voles (*Arvicola terrestris* L.) was performed. Morphology of pigment granules, their distribution along the hair layers and histology of hair bulbs were studied. Morphological data and the results of the analysis of segregation in the progeny, when brown voles were self-crossed and crossed with black ones, led to conclusion that fur colour of water voles is mediated by genes from the agouti series, precisely, black colour is determined by the extreme non-agouti allele (a^{ae} genotype), and in brown voles which are homo- or heterozygous the colour is determined by the agouti allele (AA or A^a genotypes).

- Riley PA.

Melanogenesis: a realistic target for antimelanoma therapy? *Eur J Cancer* 27:1172-1177, 1991.

Abstract : Melanin is a widely-distributed pigment in the biosphere. In the human adult, the enzymatically-catalysed process of melanin generation is the exclusive prerogative of melanocytes. Melanogenesis generates a number of reactive intermediates including orthoquinones and has been recognised as a potential hazard to melanocytes. Amplification of this cytotoxic hazard to selectively damage malignant melanogenic cells has been investigated as a rational therapeutic strategy for melanoma. A number of surrogate substrates for tyrosinase have been studied, including a range of phenols and catechols. Initial attempts to use these agents for the treatment of disseminated melanoma have foundered on problems due to unfavourable pharmacokinetics, primary toxicity or pharmacological actions of the analogue substrates, and the toxicity of hepatic metabolites. Successful

exploitation of the undoubted potential of the metabolic targeting strategy presented by the subversion of melanogenesis depends on the development of prodrugs with minimal primary toxicity and improved pharmacokinetics. The range of possible novel approaches is being extended by the emergent understanding of the complexities of melanogenesis which are outlined.

- Rosenmann A, Levin A, Neeman Z, Yanko L, Shenker JG, Rosenmann E.
Prenatal diagnosis of albinism. *Harefuah* 120:703-704, 1991.
Abstract : Prenatal diagnosis of oculocutaneous albinism (OCA) was made in 1 of 6 pregnancies at risk examined during the 20th week of gestation. A skin biopsy was taken from the fetal scalp under ultrasonic screening. Light and electron microscopy studies were performed in each case to demonstrate melanin pigment and melanosomal development in the melanocytes of the hair bulbs and the epidermis. In 1 fetus albinism was diagnosed by the absence of melanin pigment and by the demonstration that melanosomes were only present in stages I and II. In the other 5 fetuses melanin pigment and mature melanosomes (up to stage IV) were demonstrated. The pregnancy with the albino fetus was interrupted and the diagnosis of OCA was confirmed at autopsy.
- Schallreuter KU, Wood JM.
EF-hands calcium binding regulates the thioredoxin reductase/thioredoxin electron transfer in human keratinocytes and melanoma. *Novel Calcium-Binding Proteins*, 339-360. Heizmann CW. (ed), Springer, Berlin, FRG., 1991.
Abstract : A review with 32 refs. of the biochem. of thioredoxin reductase (TR)-thioredoxin (T) system; allosteric regulation of TR by Ca; the regulation of intracellular redox conditions in human keratinocytes by extracellular Ca in culture medium; Ca uptake in hypopigmentation disorders; and Ca regulation of melanin formation in human melanoma.
- Scott GA, Haake AR.
Keratinocytes regulate melanocyte number in human fetal and neonatal skin equivalents. *J Invest Dermatol* 97:776-781, 1991.
Abstract : To determine if keratinocytes influence melanocyte number and position in the developing epidermis we have experimentally recombined keratinocytes and melanocytes from epidermis of different stages of differentiation in the skin equivalent (SE) system. Previously we showed that developmental differences in the position and number of melanocytes characteristic of the epidermis in vivo were preserved in fetal and neonatal skin equivalents. In the present study we have combined cultured fetal or neonatal keratinocytes with age-matched or non-age-matched cultured melanocytes on the dermal equivalent. The ratio of basal keratinocytes to melanocytes (BK/M) present in multiple high-power fields was determined after localization of melanocytes by staining with the melanocyte-specific monoclonal antibody, HMB-45. The BK/M ratio in SE composed of neonatal keratinocytes and either fetal (n = 4) or neonatal (n = 5) melanocytes was 26.2 and 21.5, respectively. The BK/M ratio in SE composed of fetal keratinocytes and either fetal (n = 8) or neonatal (n = 5) melanocytes was 9.2 and 7.7, respectively. In each case, the BK/M ratio was dependent on the keratinocytes rather than the melanocytes. With either type of melanocyte, ratios in SE composed of neonatal keratinocytes were significantly greater than those with fetal keratinocytes. These results establish that keratinocytes regulate the BK/M ratio in this model and suggest that developmental differences between fetal and neonatal keratinocytes may be responsible for determining melanocyte numbers in the epidermal-melanin unit in vivo. The precise mechanisms that control the organization and number of melanocytes in the epidermis are unknown although keratinocytes may interact with melanocytes via growth factors, cell surface molecules, or other factors related to proliferation and differentiation of the epidermis.
- Sugumaran M.
Molecular mechanisms for mammalian melanogenesis. Comparison with insect cuticular sclerotization [corrected and republished article originally printed in *FEBS Lett* 293:4-10, 1991. *FEBS Lett* 295:233-239, 1991.
Abstract : Melanogenesis is an important biochemical process for the production of skin pigments which protect many animals from the damage of solar radiation. The abnormalities in melanogenesis

are associated with albinism, vitiligo, as well as malignant melanoma in humans. In the lower forms of animals viz., insects, the exoskeleton is hardened to protect their soft bodies by a process called sclerotization, which is often accompanied by melanization. Recent advances in the biochemistry of sclerotization and melanization reveal remarkable similarity between these two processes. The seven stages of sclerotization are: (a) enzymatic oxidation of N-acyldopamine, (b) Michael-1,4-addition reactions of N-acyldopamine quinone, (c) tautomerization of quinone to quinone methide, (d) Michael-1,6-addition of quinone methides, (e) tautomerization of N-acyldopamine quinone methide to 1,2-dehydro-N-acyldopamine, (f) enzymatic oxidation of 1,2-dehydro-N-acyldopamine, and (g) the reactions of resultant quinonoid compounds. Amazingly, striking similarities in the reaction sequences are found in the melanization process starting from dopa. These comparisons predict a central role for quinone methides as reactive intermediates during melanization. Accordingly, recent studies provide increasing evidence in favor of this proposition.

- Takahama U, Oniki T.
Participation of peroxidase in the metabolism of 3,4-dihydroxyphenylalanine and hydrogen peroxide in vacuoles of *Vicia faba* L. mesophyll cells. *Plant Cell Physiol* 32:745-754, 1991.
Abstract : When leaves of *V. faba* were treated with H₂O₂ or visible light in the presence of Meviologen (MV), the orange-red compd. dopachrome was formed transiently and melanin was accumulated. With the darkening of leaves, the level of 3,4-dihydroxyphenylalanine (DOPA) decreased and then recovered to the original level upon addn. of 1 mM H₂O₂. However, if leaves were incubated in the presence of 10 mM H₂O₂, the level of DOPA decreased again after the increase. The time course of the changes in levels of DOPA obsd. during the accumulation of melanin as a result of illumination in the presence of MV was very similar to that obsd. after the addn. of 10 mM H₂O₂. Illumination of leaves in the absence of MV did not result in any accumulation of melanin, but the level of DOPA changed slightly. When isolated mesophyll cells were incubated in the dark, the level of DOPA decreased. Illumination of the cells stimulated this decrease. Tropolone, an inhibitor of phenol oxidase, did not inhibit and actually stimulated the H₂O₂- and light-induced oxidn. of DOPA and accumulation of melanin in leaves. Tropolone also stimulated the decrease in the levels of DOPA both in the dark and in the light in isolated mesophyll cells. These data suggest that a peroxidase-H₂O₂ system, and not phenol oxidase, participates in the oxidn. of DOPA. When DOPA was oxidized by a basic peroxidase isolated from *V. faba* leaves, an intermediate, which was perhaps dopaquinone and which was reducible by ascorbate, was formed. The physiol. significance of the oxidn. of DOPA by peroxidase in vacuoles is discussed.

- Trifonov G, Ivanchev I, Apostolova A, Khristov K.
A case report of Peutz-Jeghers syndrome in childhood. *Khirurgiia (Sofia)* 44:16-19, 1991.
Abstract : The clinical course, diagnosis and treatment of Peutz-Jeghers's syndrome in childhood are discussed and a case reported in a eight-year-old girl. The disease presented with skin and mucosal melanin pigmentation in the mouth, frequent colic-like abdominal pain, due to chronic recurrent invaginations. On operation, the cause of the invaginations appeared to be 11 polypous formations in small intestines (8) and in the large intestines (3), which were subjected to radical operative removal. For an observation period the child had no complaints and was clinically healthy.

- Ueki H, Tsuda N, Kohda M, Nakagawa S.
Acquired bilateral nevus of Ota. *Hautarzt* 42:584-586, 1991.
Abstract : Two patients, a 53-year-old woman and a 73-year-old man, with a variety of a naevus of Ota (naevus fuscocoeruleus ophthalmomaxillaris) are described. In both cases, blue-brownish pigmentation appeared symmetrically on the skin of the head. Neither ocular involvement, nor nasal or oral pigmentation was found. Histological examination revealed melanin-bearing, spindle-shaped, or irregularly shaped melanocytes located exclusively in the upper dermis.

- Willershausen B, Zimmermann M, Joseph W, Fleischmann C.
Brown-black gingival hyperpigmentation--metal or melanin deposit? *Quintessenz* 42:955-963, 1991.

- Xu Y, Chen Y, Ishizu K, Li Y.
ESR studies on manganese(II) ion in Chinese limnetic pearls. Appl Magn Reson 1:283-294, 1990.
Abstract : The X-band and Q-band ESR spectra of Mn(II) ion in Chinese limnetic pearls were studied. No ESR signal of melanin-like radical was detected. A method of theor. treatment on ESR spectrum of Mn(II) ion was modified. Using it, the parameters of zero field splitting $D = 224$ Gs, $E = 74$ Gs, and $E/D = 0.33$ for Chinese limnetic pearls with bright pearlite color were calcd. The computer simulation was done using exptl. ESR spectra parameters, and satisfactory results were obtained.
- Yaar M, Gilchrest BA.
Human melanocyte growth and differentiation: a decade of new data. J Invest Dermatol 97:611-617, 1991.

3. MSH, MCH, other hormones, differentiation

- Abrao MS, Castrucci AM, Hadley ME, Hruby VJ.
Protein-kinase C mediates MCH signal transduction in teleost, synbranchus marmoratus, melanocytes. Pigment Cell Res 4:66-70, 1991.
Abstract : MCH (melanin concentrating hormone) is a heptadecapeptide, Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val, which stimulates melanosome (melanin granule) aggregation to a perinuclear position within teleost fish integumental melanocytes, resulting in lightening of the skin. The mechanisms of action of MCH are unknown. Drugs that affect the diacylglycerol/inositol triphosphate pathway were used to investigate the possible roles of this pathway in the mechanisms of action of MCH on Synbranchus marmoratus (teleost) melanocytes. The shift of the dose-response curve to MCH in the presence of various concentrations of 4-bromophenacyl bromide and neomycin sulphate, phospholipase C inhibitors, suggests that phospholipase C is stimulated after MCH receptor activation. Low concentrations (10^{-9} to 10^{-8} M) of the phorbol ester TPA exhibited MCH-like activity, eliciting a dose-dependent melanosome aggregation. Higher doses, however, displaced to the right the dose-response curve to MCH, as did the protein kinase C inhibitors, dibucaine and 1-(5-isoquinoliny)sulfonyl)-2-methylpiperazine (H-7). These results support the assumption that protein kinase C mediates the pigment aggregating activity of MCH. Both MCH and norepinephrine lightening actions were abolished by beta-glycerophosphate, a phosphatase inhibitor, suggesting that a protein dephosphorylation occurs during melanosome aggregation, and is, therefore, a common event triggered by MCH and norepinephrine, although both agonists act through separate receptors and exhibit different transduction mechanisms.
- al-Yousuf S, Mizuno N.
Electron microscopic identification of axons containing melanin-concentrating hormone in the lamprey, Lampetra fluviatilis L. Neurosci Lett 128:249-252, 1991.
Abstract : Melanin-concentrating hormone-like immunoreactivity (MCH-LI) was examined in the hypothalamo-hypophysial region of the lamprey. At the light microscopic level, MCH-LI neuronal cell bodies were shown with the peroxidase anti-peroxidase method; they were seen in the hypothalamic region close to the third ventricle. MCH-LI axons were observed to run toward the proximal neurosecretory contact region (PNCR) and the most proximal part of the neurohypophysis. Electron microscopic analysis with the immunogold method revealed that presumed secretory granules within axonal profiles showed MCH-LI. MCH-LI axonal profiles were most often seen on or near the basement membrane of connective tissue layer separating the PNCR from the hypophysial pars distalis.
- Fukuzawa T, Bagnara JT.
Control of melanoblast differentiation in amphibia by .alpha.-melanocyte stimulating hormone, a serum melanization factor, and a melanization inhibiting factor. Pigm Cell Res 2:171-181, 1989.
Abstract : A ventrally localized melanization inhibiting factor (MIF) has been suggested to play an important role in the establishment of the dorsal-ventral pigment pattern in Xenopus laevis. To examine the possibility that melanoblast expression might be controlled by local putative MIF and

melanogenic factors, the effects of .alpha.-MSH, a serum melanization factor (SMF) from *X. laevis* or *Rana pipiens*, and MIF on the outgrowth and melanization of *Xenopus* neural crest cells were studied. Outgrowth represents the no. of neural crest cells emigrating from cultured neural tubes, and melanization concerns the percentage of differentiated melanophores among the emigrated cells. MSH and SMF stimulate both outgrowth and melanization. The melanogenic effect of *Xenopus* serum in this system is more than twice that of *Rana* serum. The actions of MSH and *Xenopus* serum on melanization seem to be different. Stronger melanization is induced by *Xenopus* serum than by MSH, and the onset of melanization occurs earlier with *Xenopus* serum. MSH stimulates melanization only in the presence of added tyrosine. MSH causes young melanophores to assume a prominent state of melanophore dispersion during culture, while *Xenopus* serum (10%) had only a slight dispersing effect and not until day 3. A fraction of *Xenopus* serum presumably contg. mols. of a smaller mol. wt. (MW < 30 kDa) than that of a pigment promoting factor reported in calf serum produces the same remarkable melanogenic effects as does intact serum. While this fraction stimulates outgrowth, another fraction presumably contg. larger mols. (MS > 100 kDa) does not. MIF contained in *Xenopus* ventral skin conditioned medium (VCM) inhibits both outgrowth and melanization dose dependently. When VCM is used in combination with MSH, the stimulating effects of MSH on both outgrowth and melanization are completely inhibited. In contrast, the stimulatory effects of *Xenopus* serum are not completely inhibited when combined with VCM, although melanization is reduced to .apprx.40% that of controls. MIF activity was also found to be present in ventral, but not in dorsal, skin conditioned media of *R. pipiens* when tested in the *Xenopus* neural crest system. Ventrally localized MIF plays an important role in amphibian pigment pattern formation and the interacting effects of MIF and melanogenic factors influence melanoblast differentiation, migration, and/or proliferation of neural crest cells to effect the expression of pigmentary patterns.

- Risold PY, Fellmann D, Bugnon C.
Effect of colchicine on vasopressin and melanin-concentrating hormone neurons of the rat hypothalamus: hybridocytochemistry and immunocytochemistry studies. C.R. l'Academie Sci, Ser. III, 313:311-317, 1991.

Abstract : By 48 h after an intracerebroventricular injection of colchicine (100 .mu.g), antisera to 3 putative peptides including the rat melanin-concg. hormone (MCH) precursor, strongly stained the secretory granules accumulated in hypothalamic perikarya. In control rats, these antisera stained endoplasmic reticulum, Golgi app., or neurosecretory granules resp. Colchicine also induced a dramatic decrease in hybridization signal obtained with a probe complementary to the prepro-MCH-mRNA. Similarly, colchicine induced a strong increase in vasopressin immunoreactivity in neurons of the paraventricular and supraoptic nuclei, and a strong decrease of the vasopressin precursor mRNA. Thus, in 2 peptidergic neuron populations of the rat hypothalamus, colchicine lowers mRNAs and impairs neuropeptide protein synthesis, with respect to the accumulation of neurosecretory granules in perikarya. This paper contains an abridged English version.

4. Photobiology and photochemistry

- Andersen PH, Nangia A, Bjerring P, Maibach HI.
Chemical and pharmacologic skin irritation in man: a reflectance spectroscopic study. Contact Dermatitis 25:283-289, 1991.

Abstract : Pharmacol. and chem. in vivo skin irritation was evaluated utilizing an improved reflectance spectrophotometer equipped with computerized data anal. In 16 white females, a model for skin irritation was induced by a 24-h patch application of 4 basic chems., imipramine, norephedrine, nicotine and 8-aminoquinoline, with pKa's ranging from 3.8 to 9.5. Skin pigmentation (melanin) and the relative amts. of oxygenized (arterial) and deoxygenized (venous) Hb present in the erythematous skin were calcd. A clear increase in the Hb content was obsd. in chem. and vehicle exposed sites. Although skin irritation is a complex phenomenon involving chem. and soln. properties, percutaneous absorption and the biol. drug response, high pKa was predictive of acute skin irritation in man using computerized anal. of reflectance spectroscopy.

- Brainard GC, Stewart KT, Nguyen CD, Hanifin JP, Barker FM, Stetson MH, Hoffman RA, Rollag MD. **Mechanism for ultraviolet radiation to regulate pineal and reproductive physiology in rodents.** *Adv Pineal Res* 5:67-71, 1991.
Abstract : The mechanism for the regulation of pineal and reproductive physiol. in Siberian, albino, and Syrian hamsters by UV radiation (UVA, UVB, UVC) was studied. The UV effects were not dependent on the Harderian gland or melanin in eye but could be related to their capacity to be transmitted through the ocular lens. The results indicate that UV stimulation of the circadian and neuroendocrine system is mediated directly by one or more photopigments in the retina.

- Ho KK, Halliday GM, Barnetson RS. **Topical and oral retinoids protect Langerhans' cells and epidermal Thy-1+ dendritic cells from being depleted by ultraviolet radiation.** *Immunology* 74:425-431, 1991.
Abstract : Murine epidermis contains two types of bone marrow-derived cells of the immune system, Langerhans' cells (LC), which are dendritic antigen-presenting cells, and Thy-1+ dendritic cells (Thy-1+ DEC), which express the gamma/delta T-cell receptor for antigen and hence are probably T cells whose function in the epidermis is unknown. Ultraviolet (UV) light greatly reduces the density of both of these cell types, and hence this may be one of the mechanisms by which UV light induces immunosuppression. It is important to develop strategies for protecting these cells from the effects of UV light. In this study we show that topical all-trans-retinoic acid (RA) and an orally administered retinoid, temarotene, protect both LC and Thy-1+ DEC from being depleted by UV light. However, neither retinoid inhibited the development of immunosuppression in response to application of a contact sensitizer. We also compared two congenic mouse strains, one albino, the other lightly pigmented and capable of tanning in response to UV light. There was no difference in the ability of UV light to deplete LC or Thy-1+ DEC in these two strains or of retinoids to inhibit their depletion. These studies demonstrate that retinoids but not melanin are able to inhibit UV light from depleting LC and Thy-1+ DEC; however, there are other immunosuppressive effects of UV light which are not protected by the retinoids.

- Polewski K, Slawinska D. **Photosensitized transformation of adrenochrome.** *Physiol Chem Phys Med NMR* 23:117-123, 1991.
Abstract : The methylene blue-sensitized photooxidation of adrenochrome was studied by steady-state kinetics. The buffered, aqueous system was irradiated with light longer than 600 nm, wavelengths at which only the sensitizer absorbs. During irradiation, disappearance of adrenochrome and the formation of adrenochrome-melanin was observed. Calculated rate constants were determined on the basis of spectroscopic measurements. It was found that the observed transformation reaction steps are pH dependent. The participation of two types of photosensitized mechanism has been evidenced. Type II, singlet oxygen mechanism, predominates at pH below 9, whereas above pH 9, Type I applies. We observed the so-called "isotope effect" and a decrease of photooxidation rate in the presence of azide ion, a well-known singlet oxygen quencher, indicating the participation of singlet oxygen.

- Polewski K, Slawinska D. **Photosensitized transformation of epinephrine. I. Kinetic and spectroscopic study.** *Stud Biophys*, 139:141-148, 1991.
Abstract : The photooxidn. of epinephrine sensitized by methylene blue in aq. buffered solns. has been investigated. Irradn. has been made with light at wavelengths >600 nm, which is absorbed by the sensitizer only. During irradiation of the soln., the conversion to adrenochrome has been obsd. Prolonged irradiation leads to the appearance in the soln. of a brown, nonsol. tar, which indicates the formation of adrenomelanin. Kinetic studies allowed the calcn. of all rate consts. of these transformations. It has been found that all steps obsd. during photoconversion are pH-dependent. The appearance of green fluorescence during irradiation shows another intermediate compd. in addn. to adrenochrome. This fluorescent compd. has been identified as adrenolutin.

- Polewski K, Slawinska D. **Photochemically induced transformation of adrenochrome to adrenochrome-melanin.** *Physiol Chem Phys Med NMR* 23:125-132, 1991.

Abstract : The photochemical decomposition of adrenochrome in aqueous and deuterated solutions by visible light was investigated. From the spectroscopic study the disappearance constant $k = 4.8 \times 10(-5) \text{ s}^{-1}$ as well as quenching constant $k_q = 2.5 \times 10(-1) [\text{s M}^{-1}]$ and isotope effect $k_D/k_H = 2$ for singlet oxygen mechanism have been calculated. A possible chemical mechanism for the observed transformation of adrenochrome to the melanin polymer is discussed including the formation of the reactive intermediate species like cytotoxic quinones.

- Vermeer M, Schmieder GJ, Yoshikawa T.

Effects of ultraviolet B light on cutaneous immune responses of humans with deeply pigmented skin. *J Invest Dermatol* 97:729-734, 1991.

Abstract : The incidence of skin cancers of the basal and squamous cell types is extremely low among genetically black-skinned human beings, whereas these types of skin cancers are common among Caucasians, especially those who live in geographic areas of high sun exposure. Ultraviolet B light (UVB) is thought to be the primary oncogenic agent in sunlight. We have recently demonstrated that acute, low-dose exposure of Caucasian skin to UVB impairs the induction of contact hypersensitivity to dinitrochlorobenzene (DNCB) in approximately 40% of normal individuals. Importantly, this trait --termed UVB susceptibility-- was found to be a characteristic of virtually 100% of patients with a history of biopsy-proved skin cancer, implying that UVB susceptibility may be a risk factor for this disease. Because melanin pigment is thought to be protective of some of the deleterious effects of UVB radiation, we have examined the capacity of a low-dose regimen of UVB to alter induction of contact hypersensitivity in individuals with genetically melanized or heavily tanned skin. Our results indicate that UVB radiation depletes heavily pigmented skin of Langerhans cells, just as it does in Caucasian skin. Moreover, UVB-susceptibility exists as a polymorphic trait in individuals with genetically determined black skin, as well as in individuals with heavily tanned skin, and the incidence of this trait is similar to that found among normal Caucasian subjects. Thus, melanin does not appear to protect against the deleterious effects of an acute, low-dose regimen of UVB on induction of cutaneous immunity, and the UVB susceptibility trait is equally well-represented in both black- and Caucasian-skinned individuals. We conclude that although UVB susceptibility may function as a risk factor for skin cancer in Caucasians, it does not function similarly in black-skinned human beings, probably because melanin effectively protects against the mutagenic properties of UVB radiation.

- Young AR, Potten CS, Chadwick CA, Murphy GM, Hawk JL, Cohen AJ.

Photoprotection and 5-MOP photochemoprotection from UVR-induced DNA damage in humans: the role of skin type. *J Invest Dermatol* 97:942-948, 1991.

Abstract : Sites on previously unexposed buttock skin in 18 subjects (skin types I-V) were treated daily for 2 weeks with suberythemogenic doses of solar-simulated radiation (SSR) alone, SSR plus a UVB sunscreen, and SSR plus the same sunscreen with 5-methoxypsoralen at 30 ppm. The three sites of treatment (designated SSR, SSR/S, and SSR/S/5-MOP), and a control site that received no SSR or topical treatment, were challenged with 2MED SSR 1 week after the treatment had ceased. Biopsy samples, taken within 15 min after the challenge dose, were assessed for unscheduled DNA synthesis (UDS, interpreted as a measure of DNA damage), melanin deposition, and stratum corneum thickening. Within a given skin type, when compared with controls, the significant increase in either pigmentation or stratum corneum thickening was similar for SSR and SSR/S/5-MOP. SSR/S inhibited these endpoints. Compared with controls, UDS was significantly reduced in skin types III-V by SSR and in all skin types by SSR/S/5-MOP. SSR/S elicited no effect apart from minimal reductions in skin types IV and V. Thus, the increases in pigmentation and stratum corneum thickening seen in all skin types with SSR and SSR/S/5-MOP were accompanied by reduced UDS in all skin types with SSR/S/5-MOP but only in skin types III-V with SSR. These findings suggest that, although induced pigmentation and stratum corneum thickening may account in part for the reduction of UDS, qualitative differences in induced pigmentation may exist in skin types I-II between SSR and SSR/S/5-MOP treatments. The findings can also be interpreted to indicate that SSR/S/5-MOP treatment can afford protection against DNA damage from subsequent exposure to solar ultraviolet radiation. Risk-benefit considerations on the use of sunscreens with and without 5-MOP are discussed and the conclusion is drawn that the judicious use of 5-MOP sunscreens, particularly in skin types

I-II, affords an alternative option to those seeking a suntan.

5. Neuromelanins

- Calabrese VP, Hadfield MG.

Parkinsonism and extraocular motor abnormalities with unusual neuropathological findings. *Mov Disord* 6:257-260, 1991.

Abstract : Parkinsonian patients with ocular motility abnormalities are usually considered to have progressive supranuclear palsy. However, a number of other conditions have been noted to have the combination of parkinsonism and ocular problems. We report a case of rigid akinetic parkinsonism, oculomotor palsy, and eyelid apraxia with postmortem examination. Our findings are unusual in that there was marked gliosis of the substantia nigra with a large amount of free extracellular neuromelanin despite a 3-year clinical course. Only rare hyaline inclusion bodies and no neurofibrillary tangles were seen in the brainstem. Excessive calcification of the vessels of the globus pallidus were also noted. This case represents another example of the diversity of conditions producing parkinsonism with extraocular motor abnormalities.

- Carstam R, Brinck C, Hindemith-Augustsson A, Rorsman H, Rosengren E.

The neuromelanin of the human substantia nigra. *Biochim Biophys Acta* 1097:152-160, 1991.

Abstract : The pigment of the human substantia nigra was isolated after extraction of lipids and proteins with 2% sodium cholate in 30% ethanol followed by 2% sodium dodecyl sulfate in 10% glycerol. The pigment was hydrolysed with HI or degraded by treatment with KMNO₄ and the samples were examined for compounds known to derive from pheomelanin (4-amino-3-hydroxyphenylalanine, AHP and 4-amino-3-hydroxyphenylethylamine, AHPEA), or from eumelanin (pyrrole-2,3,5-tricarboxylic acid, PTCA). The HI hydrolysis yielded AHPEA in large quantities, indicating cysteinyl-dopamine as the main source of the pheomelanin moiety of the neuromelanin, but also trace amounts of AHP, derived from cysteinyl-dopa oxidation products. Dopamine and small quantities of dopa were also obtained by HI hydrolysis of the neuromelanin. The yield of PTCA was low, but the amounts observed show that part of the neuromelanin is of the eumelanin type, a fact compatible with an occasional exhaustion of the glutathione-cysteine reduction system at the site of neuromelanin formation.

- Hedin CA.

Smoker's melanosis may explain the lower hearing loss and lower frequency of Parkinson's disease found among tobacco smokers--a new hypothesis. *Med Hypotheses* 35:247-249, 1991.

Abstract : A new hypothesis is presented explaining the preventive effect of tobacco smoking found on noise induced hearing loss and on the frequency of Parkinson's disease. The hypothesis is based on the finding of a melanocyte stimulation of tobacco smoking in the human oral mucosa, resulting in a higher melanin content in the epithelial cells, and a higher frequency of visible oral melanin pigmentation --smoker's melanosis. The preventive influence of smoking found in the cochlea and substantia nigra may also be due to a higher melanin content and to the ability of melanin to strongly bind specific chemical agents for a long time. Melanin may in this way act as a scavenger against cell toxic factors in these organs.

- Paulus W, Jellinger K.

The neuropathologic basis of different clinical subgroups of Parkinson's disease. *J Neuropathol Exp Neurol* 50:743-755, 1991.

Abstract : Clinical and neuropathologic data in 45 patients with Parkinson's disease (PD) were compared. Twenty-seven patients suffered from marked akinesia and rigidity (AR-type) and 18 patients from predominant resting tremor (T-type). Dementia, depression, and psychosis occurred in 26, 18, and 18 patients, respectively. Neuronal counts were performed in defined areas of the medial and lateral substantia nigra (SNM, SNL), locus ceruleus (LC), and dorsal raphe nucleus (DRN). The AR-type (compared with the T-type) showed higher neuronal loss of LC, SNL, SNM, and more severe gliosis, extraneuronal melanin deposits, and neuroaxonal dystrophy in substantia nigra. Demented

PD patients showed more intense cortical Alzheimer lesions and higher neuronal depletion in the SNM, whereas PD subjects with moderate or marked dementia differed from mildly or not demented ones only in the higher degree of cortical Alzheimer lesions. More severe neuronal cell loss of DRN was observed in PD patients with depression. Occurrence of psychosis was not associated with any pathologic feature. Our findings indicate that some major clinical features of PD are related to distinct neuropathologic lesions.

- Reyes MG, Faraldi F, Senseng CS, Flowers C, Fariello R.
Nigral degeneration in acquired immune deficiency syndrome (AIDS). Acta Neuropathol (Berl) 82:39-44, 1991.

Abstract : Using stereological techniques we have estimated the volume density of melanin and counted the number of pigmented and non-pigmented neuronal cell bodies in the pars compacta of the substantia nigra of 12 autopsied patients with acquired immune deficiency syndrome (AIDS) who did not have inflammation or necrosis of the midbrain or clinical parkinsonism. The total number of neuronal cell bodies was 25% lower in AIDS (P less than 0.01) than in 12 age-matched controls, although the volume density of neuronal melanin did not differ from that of controls because the percentage of pigmented cell bodies was higher (P less than 0.01) and the cell bodies were more fully packed with melanin in AIDS. Also, the expected increase with age of the volume density of neuronal melanin (P less than 0.02) and the percentage of pigmented neurons (P less than 0.01) occurred in the controls but not in AIDS patients. Importantly, our histopathological examination showed unequivocal nigral degeneration with neuronal loss, small neuronal cell bodies packed with melanin, reactive astrogliosis and extra-cellular melanin in the AIDS patients but not in controls. Our study shows that a subclinical nigral degeneration is common in AIDS and could possibly explain the heightened susceptibility of some patients to drug-induced parkinsonism.

- Wakamatsu K, Ito S, Nagatsu T.
Cysteinyl-dopamine is not incorporated into neuromelanin. Neurosci Lett 131:57-60, 1991.

Abstract : The pigment of human substantia nigra, neuromelanin, has been thought to be derived from dopamine. To examine the genesis of neuromelanin, a new hypothesis was advanced that neuromelanin is formed by oxidn. of dopamine and cysteinyl-dopamine. On the basis of this hypothesis, synthetic neuromelanins were obtained by tyrosinase oxidn. of dopamine in the presence of various ratios of cysteine and were hydrolyzed with hydriodic acid to obtain 4-amino-3-hydroxyphenylethylamine (AHPEA). The AHPEA content in these synthetic melanins was shown to be proportional to the S content. Eleven samples of human substantia nigra were treated as well and contents of AHPEA were found to be only trace amts. These results suggest that cysteinyl-dopamine may not be incorporated into neuromelanin.

- Youdim MBH, Ben-Shachar D, Riederer P.
Iron-melanin interaction in substantia nigra as the neurotoxic component of Parkinson's disease. Adv Behav Biol 38A:257-262, 1990.

Abstract : A review with 20 refs. Topics discussed include the role of iron in oxidative stress in the substantia nigra of Parkinson's disease and iron-melanin interaction and lipid peroxidn.

6. Genetics

- Chumley FG, Valent B.
Genetic analysis of melanin-deficient, nonpathogenic mutants of *Magnaporthe grisea*. Mol Plant-Microbe Interact, 3:135-143, 1990.

Abstract : The fungus *M. grisea* causes rice blast disease and gray leaf spot disease of other grasses. Numerous *M. grisea* mutants that fail to produce the dark gray pigment typical of wild-type mycelia have been isolated and analyzed. Three classes of mutants have been distinguished based on pigmentation phenotypes: albino (Alb-), rosy (Rsy-), and buff (Buf-). Some pigment mutants were recovered following mutagenesis, and others appeared spontaneously. Spontaneous Buf- mutants have been particularly common. Genetic anal. has shown that the three mutant phenotypes are due to

single gene defects at unlinked loci. Genetic crosses have yielded the three possible classes of double mutants. Anal. of the double mutants has revealed epistasis relationships: alb- rsy- and alb- buf- mutants are Alb-, whereas rsy- buf- mutants are Rsy-. These epistasis relationships are consistent with the order of function ALB+ .fwdarw. RSY+ .fwdarw. BUF+ in melanin biosynthesis. All the pigment mutants tested failed to infect intact host plants, but the same mutants successfully infected plants that had been wounded by abrading the leaf epidermis. When scytalone, an intermediate in the biosynthetic pathway leading to dihydroxynaphthalene-based fungal melanins, was added to the growth medium, petri plate cultures of alb- mutants, but not rsy- or buf- mutants, darkened noticeably. When scytalone was incorporated in spore suspensions sprayed onto unwounded host plants, pathogenicity was restored to alb-, but not to rsy- or buf- mutants.

- Fahsold R, Rott HD, Lorenz P.

A third gene locus for tuberous sclerosis is closely linked to the phenylalanine hydroxylase gene locus. Hum Genet 88:85-90, 1991.

Abstract : Following the observation of a patient suffering from tuberous sclerosis (TSC) with a de novo reciprocal translocation t(3;12) (p26.3;q23.3), we have undertaken a linkage study in 15 TSC families using polymorphic DNA markers neighbouring the chromosome breakpoints. Significant lod scores have been obtained for markers D12S7 ($Z_{max} = 2.34$, $\theta = 0.14$) and PAH (phenylalanine hydroxylase ($Z_{max} = 4.34$, $\theta = 0.0$)). In multipoint linkage analysis, the peak lod score was 4.56 at the PAH gene locus. These data suggest the existence of a third gene locus for TSC (TSC3) on chromosome 12q22-24.1. The regions that have been found to be linked to TSC in different families map to the positions of three enzymes, phenylalanine hydroxylase (12q22-24), tyrosinase (11q14-22), and dopamine-beta-hydroxylase (9q34), all of which are involved in the conversion of phenylalanine to catecholamine neurotransmitters or melanin. Disorders of these biochemical pathways might be involved in the pathogenesis of TSC.

- Fuqua WC, Coyne VE, Stein DC, Lin CM, Weiner RM.

Characterization of melA: a gene encoding melanin biosynthesis from the marine bacterium Shewanella colwelliana. Gene 109:131-136, 1991.

Abstract : A recombinant plasmid with the ability to impart melanin synthesis to an Escherichia coli host was isolated from a Shewanella colwelliana genomic library. The genetic determinant of the Mel+ phenotype is carried on a 1.3-kb DNA fragment and sequence analysis of this revealed a single intact open reading frame that was sufficient for melanin synthesis (mel). This gene is expressed as a monocistronic transcript and a putative transcription start point is located 115 nucleotides upstream from the translational start codon. The mel gene encoded a protein of 39.5 kDa [346 amino acids (aa)] that showed no aa sequence homology with other proteins known to mediate melanin synthesis (e.g., tyrosinases).

- Iwamoto T, Takahashi M, Ito M, Hamatani K, Ohbayashi M, Wajjwalku W, Isobe K, Nakashima I.

Aberrant melanogenesis and melanocytic tumor development in transgenic mice that carry a metallothionein/ret fusion gene. EMBO J 10:3167-3175, 1991.

Abstract : This study generated four independent transgenic mouse lines that showed severe melanosis of the whole body by introducing the ret oncogene fused to the mouse metallothionein (MT)-I promoter-enhancer (MT/ret). Whereas melanogenesis was accelerated without distinct proliferative disorders in one line, melanocytic tumors frequently developed in the other three lines. Northern hybridization and in situ hybridization analyses showed that tumor cells and non-tumor melanin-producing cells expressed the transgene at high levels. The aberrant melanogenesis and tumor development were influenced by genetic and environmental factors. Furthermore, cross-breeding expts. between the transgenic mice and Wv mice suggested that the ret gene product can partially compensate for the defect of melanocyte development in Wv mice. This is a novel mammalian model in which melanosis and melanocytic tumors develop stepwise, triggered by a single transgene.

- King RA, Mentink MM, Oetting WS.

Non-random distribution of missense mutations within the human tyrosinase gene in type I

(tyrosinase-related) oculocutaneous albinism. Mol Biol Med 8:19-29, 1991.

Abstract : Type I oculocutaneous albinism (OCA) is produced by mutations of the tyrosinase gene. We report four new missense mutations in the tyrosinase gene in patients with type IA OCA. Three of these mutations occur within exon I and the fourth mutation within exon IV. Analysis of the distribution of these four missense mutations and 12 previously reported missense mutations shows that most cluster in four areas of the gene. Two clusters involve the copper A and copper B binding sites and could disrupt the metal ion-protein interaction necessary for enzyme function. The other two clusters are in exon I and exon IV and could represent important functional domains of the enzyme. We conclude that analysis of the tyrosinase missense mutations will provide insight into the structure-function relationship of this enzyme.

- Kubo Y, Nakamura H, Kobayashi K, Okuno T, Furusawa I.
Cloning of a melanin biosynthetic gene essential for appressorial penetration of Colletotrichum lagenarium. Mol Plant-Microbe Interact, 4:440-445, 1991.
Abstract : A cosmid vector (pKV.beta.) was constructed for isolating genes by complementation of mutations in *C. lagenarium*. Cosmid pKV.beta. contains the bacteriophage λ .cos site and the benomyl-resistant *C. lagenarium* .beta.-tubulin gene as a selective marker for *Colletotrichum* transformation. A genomic DNA library of wild-type *C. lagenarium* was constructed in pKV.beta.. An albino mutant strain 79215 was transformed with DNA from this library and benomyl-resistant transformants were obtained at frequencies of .apprx.20 transformants/.mu.g DNA. Seven melanin-restored transformants were obtained from .apprx.10,000 benomyl-resistant transformants. Albino mutants of *C. lagenarium* form nonmelanized appressoria and possess little penetrating ability. However, the transformants formed melanized appressoria with the ability to penetrate as efficiently as in the wild-type strain. From genomic DNA of a melanin-restored transformant, integrated cosmid sequences (pAC7) were recovered by transduction of *Escherichia coli* to ampicillin resistance following treatment *in vitro* with λ . packaging ext. Cosmid pAC7 transformed albino mutant 79215 to a melanin-restored wild phenotype with high frequency. From structural anal. of pAC7, an 8.4-kb BamHI fragment of pAC7 contains a wild-type copy of the gene involved in albino phenotype.

- Kwon BS, Chintamaneni C, Kozak CA, Copeland NG, Gilbert DJ, Jenkins N, Barton D, Francke U, Kobayashi Y, Kim KK.
A melanocyte-specific gene, Pmel 17, maps near the silver coat color locus on mouse chromosome 10 and is in a syntenic region on human chromosome 12. Proc Natl Acad Sci U S A 88:9228-9232, 1991.
Abstract : Melanocytes preferentially express an mRNA species, Pmel 17, whose protein product cross-reacts with anti-tyrosinase antibodies and whose expression correlates with the melanin content. We have now analyzed the deduced protein structure and mapped its chromosomal location in mouse and human. The amino acid sequence deduced from the nucleotide sequence of the Pmel 17 cDNA showed that the protein is composed of 645 amino acids with a molecular weight of 68,600. The Pmel 17 protein contains a putative leader sequence and a potential membrane anchor segment, which indicates that this may be a membrane-associated protein in melanocytes. The deduced protein contains five potential N-glycosylation sites and relatively high levels of serine and threonine. Three repeats of a 26-amino acid motif appear in the middle of the molecule. The human Pmel 17 gene, designated D12S53E, maps to chromosome 12, region 12pter-q21; and the mouse homologue, designated D12S53Eh, maps to the distal region of mouse chromosome 10, a region also known to carry the coat color locus *si* (silver).

7. Tyrosinase and other enzymes

- 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 examined in hair follicles from twenty three 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 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 phaeomelanin content in the hair but in the dark-haired individuals a better correlation was seen with eumelanin. α -Melanocyte stimulating hormone 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. 8-Bromo-cyclic AMP, on the other hand, increased tyrosinase synthesis but only in the hair follicles from dark-haired subjects. These findings contrast with those previously reported in mice and it would appear that the control mechanisms that regulate tyrosinase in human melanocytes are different in many respects from those in mice.

- Burchill SA, Bennett DC, Holmes A, Thody AJ.
Tyrosinase expression and melanogenesis in melanotic and amelanotic B16 mouse melanoma cells. Pathobiology 59:335-339, 1991.
Abstract : Tyrosinase activity, abundance, and mRNA transcription were examined in three sublines of the B16 mouse melanoma. Tyrosinase activity and melanin content were highest in the B16-F1 cells, slightly less in the B16-F10, and markedly lower in the B16-F10-DD cells. No differences in the level of tyrosinase mRNA or protein were found in the three different sublines. Thus, the differences in tyrosinase expression arise from the post-translational modification of the enzyme causing its activation or inhibition.
- Chakraborty AK, Ichihashi M, Mishima Y.
Effect of DOPA-loading on glutathione metabolizing enzymes and tyrosinase in relation to 5-S-cysteinyl-DOPA formation in cultured B-16 melanoma cells. J Dermatol Sci 2:329-335, 1991.
Abstract : The effects of DOPA and GSH were detd. on enzyme systems for 5-S-cysteinyl-DOPA (5SCD) formation in murine melanoma cells cultured in tyrosine- and cystine-free medium. DOPA at its optimum concn. (10^{-5} M) when added alone did not alter tyrosinase, glutathione-S-transferase (GST) or γ -glutamyl transpeptidase (γ -GTP) activities. In the presence of GSH at its optimum concn. (10^{-5} M), DOPA loading did not change tyrosinase or glutathione S-transferase activities. This indicates that the higher 5SCD levels obsd. in the medium because of DOPA loading in the GSH-dependent system result from increased substrate availability rather than the increased enzyme activity. An acute drop in 5SCD at DOPA concns. above 10^{-5} M obsd. in the GSH-dependent system may be due to the inhibition of tyrosinase at high substrate concns. (10^{-4} M). Conversely, in the presence of DOPA, when GSH was increased, the resultant higher prodn. of 5SCD could be explained by the increased activity of GST. When added alone, GSH (10^{-5} M) caused an increase in GST (.apprxq.50%) activities. A drop in 5SCD in the medium when GSH was added beyond its optimum concn. (10^{-5} M) in the DOPA-dependent system could be due to competitive inhibition of γ -GTP by GSH. The data demonstrate that 5SCD formation may be enhanced due to the accumulation of cytotoxic melanin precursors such as DOPA/DOPA quinone. The relative quantities of GSH at the sites of DOPA quinone formation and the levels of its metabolizing enzymes can influence the type of product formed. Although this explains the importance of GST in elevated 5SCD prodn., the inverse relation of γ -GTP with 5SCD indicates a complex regulatory influence of melanin precursors on this enzyme in melanoma cells.
- Chen JS, Wei CI, Marshall MR.
Inhibition mechanism of kojic acid on polyphenol oxidase. J Agric Food Chem 39:1897-1901, 1991.
Abstract : The mechanism of inhibition of polyphenol oxidase (PPO) of mushroom, potato, apple, white shrimp, and spiny lobster by kojic acid was investigated. Polarog. indicated that kojic acid inhibited melanosis by interfering with the uptake of O required for enzymic browning. Spectrophotometry and chromatog. demonstrated that kojic acid was capable of reducing o-quinones to diphenols to prevent the final pigment (melanin) forming. The preincubation temp. did not significantly affect PPO inhibition by kojic acid.
- Geremia E, Corsaro C, Baratta D, Santoro C, Scalia M, Sichel G.
Antioxidant enzymic systems in pigment tissue of amphibia. Pigm Cell Res 2:208-212, 1989.

Abstract : Superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities in pigmented and unpigmented liver tissues of frog and albino rat, resp., were studied. Pigmented tissue is lacking in manganese superoxide dismutase activity, and the main enzymic activity utilized in the cytosol by pigmented cells to reduce the hydrogen peroxide to water is represented by catalase; on the contrary, for the same reaction, the cells of albino rat liver primarily utilize glutathione peroxidase activity. Both a low glutathione peroxidase activity and a low glutathione reductase activity were found in pigmented tissue of frog liver when compared with unpigmented tissue of rat liver. The authors believe that, in pigmented cells, melanin could act as a reducing physiol. agent replacing glutathione in the redn. of hydrogen peroxide. This reducing action of melanin could cause a diminished need for GSH and, therefore, could provoke the low glutathione peroxidase and reductase activities in pigmented tissue.

- Li J, Nappi AJ.

Electrochemical identification of dopachrome isomerase in *Drosophila melanogaster*. Biochem Biophys Res Commun 180:724-729, 1991.

Abstract : A principal reaction in the eumelanin biosynthetic pathway is the conversion of dopachrome (DC) to dihydroxyindole(s). Dopachrome isomerase (DI), the enzyme that catalyzes this reaction, was detected for the first time in larvae of *D. melanogaster*. Unlike the enzyme from B16 mouse melanoma cells which converts dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA), the insect enzyme forms 5,6-dihydroxyindole (DHI). The activity of the insect DI was linear through 15 min incubation, and the amount of DHI produced was proportional to the amount of enzyme that was incorporated into the reaction mixtures.

- McEwan MT, Parsons PG.

Regulation of tyrosinase expression and activity in human melanoma cells via histamine receptors. J Invest Dermatol 97:868-873, 1991.

Abstract : In cultured human melanoma cells, histamine H1 (mepyramine) and H2 receptor antagonists (cimetidine, ranitidine, impromidine) increased tyrosinase activity, whereas H2 agonists (dimaprit, nordimaprit) decreased activity. Mixtures of agonist and antagonist either decreased or increased tyrosinase activity, depending on the relative concentrations of each drug. Nordimaprit, the most effective inhibitor, lowered tyrosinase activity significantly within 36 h and caused a slower loss of tyrosinase protein as judged by reactivity with two monoclonal antibodies. Prolonged treatment of a melanotic cell line with nordimaprit led to complete loss of pigment, with no loss of the 56-kDa melanosomal antigen 1C11. Cells remained amelanotic for 8 weeks after removal of the drug and, even after 26 weeks, melanin content and tyrosinase expression and activity had not fully recovered. Nordimaprit increased the rate of degradation of tyrosinase and of nordimaprit binding proteins. Whereas nordimprit did not directly inhibit tyrosinase, lysates of treated cells contained an inhibitory activity that partitioned approximately equally across a 10-kDa ultrafiltration membrane. Overall, these results showed that melanogenesis can be controlled via histamine receptors, the mechanism for the H2 agonist nordimaprit consisting of three components: induction of a tyrosinase inhibitor, increased degradation of tyrosinase, and long-term down-regulation of tyrosinase expression.

- Naeyaert JM, Eller M, Gordon PR, Park HY, Gilchrest BA.

Pigment content of cultured human melanocytes does not correlate with tyrosinase message level. Br J Dermatol 125:297-303, 1991.

Abstract : Tyrosinase is considered to be the rate-limiting enzyme for the biosynthesis of melanin in epidermal melanocytes, and thus tyrosinase activity is thought to be a major regulatory step in melanogenesis. To determine whether the rate of pigment production was controlled at the level of tyrosinase gene expression, we developed a culture system capable of generating large populations of pure human melanocytes and then measured both melanin content as determined spectrophotometrically by absorption at 475 nm and mRNA levels as detected by hybridization with cloned cDNA Pmel 34, encoding human tyrosinase. We examined the relationship between pigment content and tyrosinase mRNA levels among human melanoma and melanocyte lines with very different levels of basal pigmentation; between two clones of a single human melanoma line, one pigmented and one amelanotic; and sequentially in melanocytes before and after simulation with

isobutylmethylxanthine to increase melanin content per cell. Using Northern blot analysis and in-situ hybridization we found no correlation between tyrosinase message levels and melanin content, suggesting that posttranscriptional regulation of tyrosinase and/or other events determine the rate of pigment synthesis in human melanocytes.

- Nappi AJ, Seymour J.
Hemolymph phenol oxidases in *Drosophila melanogaster*, *Locusta migratoria*, and *Austropotamobius pallipes*. *Biochem Biophys Res Commun* 180:748-754, 1991.
Abstract : The early enzyme-mediated reaction sequence in the biosynthesis of melanin from L-tyrosine involves an initial hydroxylation (monophenol oxidase activity, MPO) of the aromatic amino acid precursor to form L-dopa (3,4-dihydroxyphenylalanine), and the ensuing oxidation (diphenol oxidase activity, DPO) of the resultant diphenol to form dopaquinone. By means of high pressure liquid chromatography with electrochemical detection (HPLC-ED) both phenol oxidase activities were observed in the blood (hemolymph) of two species of insect, third-stage larvae of *Drosophila melanogaster* and adult *Locusta migratoria*, and in an adult fresh-water crayfish, *Austropotamobius pallipes*. These results establish that in each species MPO and DPO can be detected readily without the use of exogenous activators.
- Schallreuter KU, Wood JM, Berger J.
Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol* 97:1081-1085, 1991.
Abstract : Suction blister roofs taken from the involved and uninvolved epidermis of patients with vitiligo showed a consistent reduction in levels of catalase compared to normal healthy controls of matched photo-skin types (Fitzpatrick classification). A decrease in catalase activity is expected to increase the concentration of hydrogen peroxide in the epidermis of these patients. Hydrogen peroxide functions as a reversible inhibitor of human tyrosinase with a KI of 8×10^{-6} M. Also, hydrogen peroxide undergoes photochemical reduction yielding highly reactive hydroxyl radicals (OH \cdot) and hydroxyl ions (OH $^-$) mainly by the Haber-Weiss reaction. Hydroxyl radicals are capable of bleaching constitutional melanin and cause membrane lysis through lipid peroxidation reactions. Hydroxyl ions increase the pH in the epidermis, and as a consequence glutathione reductase activity is increased in patients with vitiligo compared to controls. Based on these new results, together with the previously reported calcium transport defect, a new hypothesis has been formulated for the pathogenesis of vitiligo.

8. Melanoma and other pigmented tumours

- Chetty R.
Pigmented adrenal pheochromocytomas. *Hum Pathol* 22:1180, 1991.
- Datubo-Brown DD.
Primary malignant skin tumors in Nigerians. *J Natl Med Assoc* 83:345-348, 1991.
Abstract : Skin cancers, although uncommon, do occur in black Africans. Available literature on this subject from black African populations is scant, suggesting diminished interest. Eighteen cases of malignant skin tumors seen at the University of Port Harcourt Teaching Hospital over 3 years (1984 to 1987) were analyzed for diagnoses, site of tumors, sex, and age. Seven patients (39%) had malignant melanomas affecting only the soles of the feet, while the same number had squamous cell carcinomas widely distributed in various parts of the body. Basal cell carcinomas were found in four (22%) patients with face lesions. Only three albinos were in the series, and all three had squamous cell carcinomas. Melanin protection against sun-induced skin cancers gives a false sense of well-being. The need for renewed interest of the subject is emphasized.
- Edward M, MacKie RM.
Pentoxifylline enhances lung colonization and alters cell adhesion and glycosaminoglycan synthesis by metastatic B16 melanoma cells. *Int J Cancer* 49:711-716, 1991.
Abstract : The effect of pentoxifylline on B16 melanoma cell lung colonization, synthesis and

properties of glycosaminoglycans (GAGS), and adhesion to and degradation of subendothelial extracellular matrix was examined. Pentoxifylline inhibited cell growth, cell numbers being reduced by 50% following incubation for 4 days in the presence of 250 micrograms/ml pentoxifylline, while the treated cells appeared more flattened, possessed numerous but short dendritic processes, and exhibited greatly enhanced tyrosinase activity and melanin synthesis. Pentoxifylline treatment increased the cells' ability to colonize the lungs of syngeneic C57BL mice following tail-vein injection of 10(5) cells. The number of lung tumours increased from 16.7 +/- 6.1 to 52.2 +/- 17.8. In addition, pentoxifylline-treated cell GAG synthesis was reduced by 36%, and the charge density of chondroitin sulphate reduced, while tumour-cell aggregation and adhesion to subendothelial extracellular matrix was increased, as was the tumour-cell-mediated release of $^{35}\text{SO}_4$ from radiolabelled subendothelial matrix. The observed changes in GAG synthesis may contribute toward the increased cell adhesiveness which, in addition to increased degradation of certain components of the subendothelial extracellular matrix, may account, at least in part, for the enhancement of lung colonization.

- Eri Z, Stanic J, Stanic M.

Primary malignant melanoma of the trachea. *Plucne Bolesti* 43:75-77, 1991.

Abstract : The paper gives a case report of the primary malignant melanoma of the trachea in a 61 years old patient with micrometastasis in the right upper lobe of the lung. The diagnosis was made after autopsy. Macroscopically, a polypoid, greyish, partly dark brown tumor was found 6 cm above the tracheal bifurcation on the site of connection between the membranous and cartilaginous part. The tumor was fixed to the trachea wall with a very narrow long stalk, causing long dispnoic attacks worsening in a back lying position particularly. Histologically, both the primary tumor and its secondary deposit in the right lobe were found to have similar appearance as a malignant melanoma elsewhere. Melanin pigment was found in abundance here and there and easily detected already in preparations stained by the hematoxylin-eosin method. The large polypoid tumor caused an obstruction of the trachea and finally suffocation.

- Foley GL, Valentine BA, Kincaid AL.

Congenital and acquired melanocytomas (benign melanomas) in eighteen young horses. *Vet Pathol* 28:363-369, 1991.

Abstract : In a retrospective study, cutaneous melanocytic tumors from 18 horses, less than 2 years old, were examined histopathologically and clinical follow-up requested. Melanocytomas (benign melanomas) occurred in a variety of breeds and in horses of varied coat color. The age of the horses at the time of biopsy ranged from 3 weeks old to 2 years old. Four melanocytomas were congenital, 11 melanocytomas were acquired by 1 year of age, and three were acquired prior to 2 years of age. Of the 18 horses, five were male, and 13 were female. All tumors were solitary and located on the legs or trunk; none were in the perineal region. Ulceration of the overlying epidermis was common. Tumors were generally localized and were not encapsulated. The tumors had a variety of cell patterns ranging from sheets, to streams, or nests of melanocytes. Cellular morphologic findings also ranged from epithelioid, to a mixture of epithelioid and spindle cells or to a spindle pattern. The nuclei were large and euchromatic, especially in the epithelioid cells. Several tumors had moderate cellular pleomorphism and binucleate cells. Mitotic activity was generally low (less than 1/high-powered field), but was readily detected (1-2/high-powered field) in bleached sections of four cases. Melanin pigmentation varied from mild to heavy. Melanophages were admixed with the tumor cells or in the adjacent tissue. Follow-up information was obtained on 15/18 horses and revealed that 14/15 horses were free of recurrence following excision. One neoplasm, that was poorly demarcated and had a spindle cell pattern, was not completely resected and continued to grow. These melanocytic tumors in young horses are distinct from melanomas in aged horses in their location, epithelial involvement, and age of horses affected. The majority of these tumors appear to be benign and share features of melanocytic nevi of human beings.

- Franquemont DW, Mills SE.

Sinonasal malignant melanoma. A clinicopathologic and immunohistochemical study of 14 cases. *Am J Clin Pathol* 96:689-697, 1991.

Abstract : The clinical, light microscopic, and immunohistochemical features of 14 sinonasal malignant melanomas were studied to show their diverse morphologic appearance and distinction from therapeutically more amenable neoplasms that occur in this region. The tumors arose in 6 men and 8 women (median age, 70 years). Eleven patients died of disease 7 to 44 months (median, 18 months) after diagnosis. The absolute median survival time was 18.5 months (range, 7 to 44 months). The predominant microscopic appearance was categorized as small blue cell in eight cases, spindle cell in three cases, epithelioid in two cases, and pleomorphic in one case. Eight tumors had multiple patterns. Five sinonasal malignant melanomas had theque-like growth, five had junctional change, and 10 contained at least rare melanin pigment. Fourteen, 13, and 12 sinonasal malignant melanomas were immunoreactive with anti-vimentin, HMB45, and anti-S100 protein antibodies, respectively. One epithelioid tumor positive for vimentin, S100, and HMB45 also contained scattered epithelial membrane antigen-positive and cytokeratin-positive cells, which emphasizes the need for a battery of stains to distinguish sinonasal malignant melanoma from carcinoma. All tumors were negative for leukocyte common antigen, muscle-specific actin, and synaptophysin. Diffuse immunopositivity for vimentin, S100 protein, and HMB45 allows distinction of sinonasal malignant melanomas from histologically similar neoplasms.

- Gratz KW, Makek M, Sailer HF.

Malignant melanotic schwannoma of the oral cavity. Int J Oral Maxillofac Surg 20:236-238, 1991.

Abstract : Intraoral malignant melanotic schwannoma is an extremely rare tumor. Two cases are presented, one occurring in the mandible of a 62-year-old man, the other in the maxilla of a 79-year-old man. The clinical presentation, light microscopic findings and immunohistopathological features are described. The difficulty of diagnosing this special tumor at initial presentation correctly, is discussed.

- Moritomo T, Saito H, Watanabe T, Mochizuki K.

Tissue culture study on Mongolian gerbil's (*Meriones unguiculatus*) malignant melanoma. Jikken Dobutsu 40:385-388, 1991.

Abstract : We tried to culture melanoma cells from a Mongolian gerbil's (*Meriones unguiculatus*) malignant tumor. In primary culture, most of cells have abundant melanin granules in their cytoplasm. Melanin granules decreased through 5 to 15 serial passages and disappeared after 15 th passage. The morphology of the cells varied from spindle to large polydendritic cells. Although typical melanin granules were not seen when the cells were stained by Masson-Fontana method, the cells were positive for DOPA reaction. Electron microscopically, most of the cells have well-developed Golgi apparatus and rough-surfaced endoplasmic reticulum with dilated cistern, and premelanosome-like granules were frequently observed in their cytoplasm.

- Wollina U, Kilian U, Henkel U, Schaarschmidt H, Knopf B.

The initial steps of tumor progression in melanocytic lineage: a histochemical approach. Anticancer Res 11:1405-1414, 1991.

Abstract : Antigen expression was studied by immunohistochemistry in 133 human melanocytic skin lesions to gain insight into the initial steps of tumor development, i.e. in particular the change from melanocytes to benign nevi. We refer to the proposed progression model of Clark and co-workers. The following types of antigens were investigated: (i) intermediate filament antigens (vimentin), (ii) melanoma-associated antigens (HMB-45, NKI/C3, MA-930, LS59), (iii) proliferation-associated antigens (S-100, Ki67, Ro/SSA, calmodulin), (iv) progression-associated antigens (HLA-DR, ICAM-1), and (v) basal membrane antigens (bullous pemphigoid antigen, laminin, fibronectin, collagen type IV). The intensity of expression and the topography of immunoreactive pigment cells were compared with the stage of tumor progression. Special attention was paid to the early steps of this process, i.e. the disturbance of the epidermal melanin unit and the development of melanocytic ("nevocellular")nevi. A dramatic shift of antigen expression (antigen types [i] to [v]) was noted in benign nevi compared with melanocytes. Nevi with cellular atypia disclosed a tendency towards an increased percentage of tumor cells reactive for melanoma- and progression-related antigens (types [ii] and [iv]). However, there was no clear cut level of distinction of antigen expression (types [i] to [v]) between benign and primary malignant melanocytic tumors. So-called dysplastic nevi resembled

benign tumors or melanocytes rather than malignant melanoma. Metastatic melanoma of skin showed a relatively high number of Ki67-positive, cycling melanoma cells. The results have a bearing on the concepts of melanocytic nevus ontogenesis and "maturation". It appears that melanocytes lose maturity on their way down to the dermis in contrast to traditional concepts (Abtropfung); this might be of importance for our understanding of melanoma development in association with melanocytic nevi. Our findings are discussed with regard to Clark's model of tumor progression.

- Yoshitomi K, Boorman GA.

Spontaneous amelanotic melanomas of the uveal tract in F344 rats. Vet Pathol 28:403-409, 1991.

Abstract : Five intraocular amelanotic melanomas were identified in the National Toxicology Program's database consisting of records from more than 60,000 female and 60,000 male F344 rats, which were used as control and treated animals in 2-year carcinogenicity studies. The five spontaneous melanomas were grossly observed as white or yellow, unilateral nodules, which originated in the region of the iris and ciliary body, often also involving the choroid. These amelanotic melanomas were composed predominantly of spindle cells arranged in a whorled pattern often with perivascular orientation. Mitotic figures were common in five tumors. The spindle cells had a positive immunoreactivity for S-100 protein but were negative for desmin. Electron microscopic studies provided clear evidence that these tumors originated from the uveal melanocytes. Ultrastructurally, the spindle cells contained numerous cytoplasmic premelanosomes (stage II melanosomes) that were not associated with melanin. Special histochemical studies showed that the spindle cells had a negative reaction for melanin. Although electron microscopic features are critical in the diagnosis of amelanotic melanomas of the uveal tract, the whorled pattern of spindle cells is a useful histologic criterion in differential diagnosis of this tumor in F344 rats.

- Young RH, Scully RE.

Malignant melanoma metastatic to the ovary. A clinicopathologic analysis of 20 cases. Am J Surg Pathol 15:849-860, 1991.

Abstract : Twenty cases of malignant melanoma metastatic to the ovary are reported. The patients, whose ages ranged from 21 to 60 (average 37.5) years, typically presented because of abdominal swelling or pain. Approximately 50% of the patients also had metastatic tumor outside the ovary, usually within the pelvis and upper abdomen, at the time of presentation. Twelve patients were known to have had a cutaneous malignant melanoma 1 month to 13 years before their ovarian tumors were discovered, and pigmented lesions had been removed previously from three other patients. Most patients are known to have died within a few years of discovery of their ovarian tumors but two were alive without evidence of disease 5 and 8 years later. The ovarian tumors, which were bilateral in nine cases, ranged up to 20 (average 10.5 cm) in greatest dimension. Six of them were either entirely black or had discernible black or brown foci. The most common microscopic appearance was that of large cells with abundant eosinophilic cytoplasm growing in nodular aggregates or diffusely. Occasional tumors were characterized by small cells with scanty cytoplasm, and in five tumors spindle cells were present. Another pattern was growth in the form of discrete rounded aggregates having a nevoid appearance. Eight tumors contained folliclelike spaces. Major cytologic features of the tumors included prominent nucleoli in 13, cytoplasmic pseudo-inclusions in many nuclei in five, and intracytoplasmic melanin pigment in nine cases. In the 10 cases studied immunohistochemically, most of the tumor cells were strongly positive for S-100 protein and fewer cells were positive for HMB-45 in the seven tumors that were stained for this antigen. Melanosomes were identified in the three tumors examined ultrastructurally. These neoplasms often were difficult to differentiate from many other types of tumors, including juvenile granulosa cell tumor and small cell carcinoma, because of the presence of folliclelike spaces.

9. Eye

- Barrenas ML, Lindgren F.

The influence of eye colour on susceptibility to TTS in humans. Br J Audiol 25:303-307, 1991.

Abstract : In order to investigate the function of the inner ear melanin, noise-induced temporary

hearing loss (temporary threshold shift, TTS) was studied in humans with either blue or brown iris colour. Sixty-eight normally hearing teenage boys participated in this study. Hearing thresholds before and after exposure were established with a computerized sweep frequency audiometer in the frequency range 0.8-8 kHz. The noise exposure consisted of a 1/3 octave band-filtered noise with centre frequency 2 kHz at 105 dB SPL for 10 min. The mean TTS in the frequency range 2-8 kHz showed a significant difference with the brown-eyed subjects developing least TTS, and the blue-eyed subjects most TTS.

- Baudouin C, Brignole F, Bayle J, Fredj-Reygrobeller D, Lapalus P, Gastaud P.

Immunomorphological study of the vitreous body and subretinal fluid in vitreoretinal proliferation. J Fr Ophtalmol 14:147-152, 1991.

Abstract : Proliferative vitreoretinopathy (PVR) is a major complication of rhegmatogenous retinal detachment. Its pathogenesis remains poorly understood and the accurate nature of the growing cells on both surfaces of the detached retina has not been yet determined. We undertook an immunocytological study on 28 specimens of vitreous or subretinal fluid removed from patients with PVR. Five main types of cells could be identified: heavily pigmented cells, poorly pigmented ones, large totally unpigmented macrophage-like ones, smaller unpigmented cells and lymphocytes. Analysis of intravitreal pigment granules showed two different types of pigmented cells, those with lipofuscin and melanin and those with melanin without any granules of lipofuscin, which could originate from ciliary or iris pigment epithelia. Immunostaining procedures confirmed the epithelial non macrophagic lineage of the intravitreal and subretinal cells. Lymphocytes were only B cells. These results confirm the importance of proliferative process during the course of PVR and shows the involvement of other ocular structures other than the retinal pigment epithelium.

- Baudouin C, Hofman P, Brignole F, Bayle J, Loubiere R, Gastaud P.

Immunocytology of cellular components in vitreous and subretinal fluid from patients with proliferative vitreoretinopathy. Ophthalmologica 203:38-46, 1991.

Abstract : Proliferative vitreoretinopathy accounts for most of failures in retinal detachment surgery. It results from the formation of membranes spreading onto inner and outer surfaces of the detached retina and within the vitreous body, but the nature of the growing cells and the mechanisms of proliferation remain speculative. A cytological study was thus undertaken on 35 specimens of vitreous and subretinal fluid obtained surgically in patients with proliferative vitreoretinopathy. Various types of cells were identified: typical pigment epithelial cells, lightly pigmented and large totally unpigmented macrophage-resembling cells, smaller unpigmented cells and lymphocytes. Immunocytological procedures with 10 different monoclonal antibodies directed against different markers of epithelial and immunocompetent cells showed the epithelial nonmacrophagic origin of the intravitreal and subretinal cells, as most of these cells were positive for cytokeratin but remained negative for macrophage markers. Examination of intravitreal pigment granules, using autofluorescence analysis by epi-illumination and toluidine blue staining, showed two distinct populations of pigmented cells, one containing melanin and the other lipofuscin, suggesting that pigmented cells could originate from the retinal and ciliary pigment epithelia. As concerns lymphocyte identification, only B cells were seen, whereas no T lymphocyte could be found. Fibronectin was found on a minority of cells in 4 vitreous specimens, but cells positive for glial fibrillary acidic protein could not be seen. These results confirm the involvement of pigment epithelial cells and the strong morphological changes they undergo during the course of proliferative vitreoretinopathy, but the mechanisms of proliferative phenomena after retinal detachment remain to be determined.

- Haller EM, Langmann G, Schwab C.

Histology and transmission electron microscopy of the cornea in xeroderma pigmentosum type C. Graefes Arch Clin Exp Ophthalmol 229:395-400, 1991.

Abstract : Xeroderma pigmentosum is a very rare precancerous skin disease that is triggered by sunlight. It is caused by a defect in the DNA repair system and causes benign and malignant transformations. Only eye tissues that come into contact with UV light are affected, such as the lids, conjunctiva and cornea. We describe a patient who suffered from xeroderma pigmentosum type C,

showing the typical skin alterations but no sign of malignancy. A perforating keratoplasty was performed on both eyes because of the dense opacity of the corneas. The corneal buttons obtained were examined by light and transmission electron microscopy. Degeneration was found only in the basal-cell layer of the corneal epithelium. The most severe morphological changes were seen in Bowman's layer, the subepithelial stroma, Descemet's membrane and the corneal epithelium. Bowman's layer was often interrupted or replaced by a degenerative pannus, which extended into the underlying stroma. Subepithelial "channels" were localized in the basal epithelium and protruded into the subepithelial stroma. In both corneas, Descemet's membrane contained different amounts of so-called lattice collagen, and the remaining endothelial cells in the left cornea contained numerous melanin granules.

- Hirota A, Mishima H, Kiuchi Y, Nagata A, Kurokawa T, Ishibashi S.
Effect of topical 8-hydroxy carteolol on intraocular pressure and melanin granules. Nippon Ganka Gakkai Zasshi 95:752-757, 1991.
Abstract : It was reported that 8-Hydroxycarteolol (8-OH CA), the major metabolite of carteolol hydroxychloride (CA), has a slightly different pharmacological effect from CA. We studied the reduction of intraocular pressure (IOP) on a single eyedrop application of 8-OH CA in albino and pigmented rabbit eyes. To determine the characteristic of 8-OH CA and CA, we investigated the binding ability of these drugs to synthetic melanin. In the present study, topically applied 2% CA did not significantly decrease IOP in albino and pigmented rabbit eyes. Topically applied 0.01, 0.05, 0.1 and 1.0% 8-OH CA significantly decreased the IOP of albino rabbit as did 0.1 and 1.0% 8-OH to pigmented rabbit eyes. The maximum reduction of IOP was 3.5 +/- 0.33 mmHg (mean +/- SEM) in albino rabbit and 3.0 +/- 0.45 mmHg (mean +/- SEM) in pigmented rabbit. Maximum IOP reduction was obtained after 30 min. from topical application in albino rabbit, but in pigmented rabbit after 1 hour or later. Our binding studies to melanin show that the melanin binding ability is less for 8-OH CA than for CA at any concentrations. These results may indicate that lower concentrations of topically applied 8-OH CA profoundly reduce IOP compared to CA, and 8-OH CA has less effect on melanin than CA.

- Kaidbey KH, Barnes A.
Determination of UVA protection factors by means of immediate pigment darkening in normal skin. J Am Acad Dermatol 25:262-266, 1991.
Abstract : A method is described for screening potentially useful photoprotective agents against UVA radiation by the use of immediate pigment darkening as an end point. Threshold doses of immediate pigment darkening showed a log normal distribution and the response was found to obey dose-reciprocity at irradiance levels below 50 mW/cm². With this procedure, several marketed sunscreens containing benzophenone-3 as the only UVA absorber were found to have poor UVA protection factors, whereas those containing combinations of benzophenone-3 and butyl methoxydibenzoyl methane or melanin were more effective. There was no correlation between the sun protection factor cited on the label and the calculated immediate pigment darkening-protection factor.

- Nagata A.
Interaction of antiglaucomatous drugs and melanin granule. Nippon Ganka Gakkai Zasshi 95:644-649, 1991.
Abstract : The author investigated the effects of several antiglaucomatous drugs and prostaglandins (E2 and F2 alpha) on intraocular pressure responses in pigmented and albino rabbits, and spectrophotometrically assessed the binding of these drugs to synthetic melanin. Topical application of 0.5% timolol, 1% epinephrine and 3% pilocarpine had greater ocular hypotensive effects in albino rabbits than in pigmented rabbits. However no such differences were seen with application of prostaglandins. Melanin binding of drugs was higher in the order of befunolol, carteolol, timolol, epinephrine and pilocarpine. At higher concentrations of the drugs, the degree of binding increased. Prostaglandins had no binding ability. Thus drugs with lower hypotensive effects in pigmented rabbits than in albino rabbits had high melanin binding ability, whereas drugs with similar effects in pigmented and albino rabbits had no melanin binding ability. It is speculated that some antiglaucomatous drugs bind to melanin, resulting in decreased pharmacological action.

- Oguni M, Tanaka O, Shinohara H, Yoshioka T, Setogawa T.
Ultrastructural study on the retinal pigment epithelium of human embryos, with special reference to quantitative study on the development of melanin granules. Acta Anat (Basel) 140:335-342, 1991.
Abstract : The development of the retinal pigment epithelium (RPE) was studied ultrastructurally, using 13 externally normal human embryos, Carnegie stages ranging from 13 to 23 (4-8 week of gestation). Melanosomes in the peripheral and posterior RPE were classified according to Fitzpatrick et al. The melanosome of phase I is formed from the Golgi complex and parcelled off into small vesicles. The vesicle enlarges and elongates to form an oval organelle with membranous structures in it (phase II melanosome). Subsequently, melanin deposits on the membranous structures of the melanosomes (phase III melanosomes), and the completion of this process produces a uniformly electrondense granule without discernible internal structures (phase IV melanosome). Melanosomes of phases III and IV appeared in the RPE at stage 15. As the embryonic stage advanced, the ratio of phase II melanosomes decreased and that of phase IV melanosomes increased. The number of phase III melanosomes reached a peak in the peripheral and posterior RPE at stages 15 and 18, respectively. After stage 17, the increase in melanosomes and intracellular organelles was more prominent in the posterior than in the peripheral RPE. During stages 13 and 15, gap junctions were present not only in the apical but also basal plasma membranes of the RPE. At stage 20, gap junctions in the basal plasma membrane disappeared except for the transitional areas from the RPE to the neural retina (NR). In addition, gap junctions were observed between NR and RPE only in the peripheral region at stage 20. The morphological and quantitative differences in the peripheral and posterior RPE in the embryonic period are discussed.
- Summers CG, Creel D, Townsend D, King RA.
Variable expression of vision in sibs with albinism. Am J Med Genet 40:327-331, 1991.
Abstract : Oculocutaneous albinism is defined by the presence of cutaneous and ocular hypopigmentation, the latter associated with nystagmus, iris transillumination, reduced retinal pigment, foveal hypoplasia, and misrouting of the optic fibers at the chiasm. The visual acuity is variable but almost always reduced. We report on two brothers with oculocutaneous albinism and markedly different visual acuity. One brother has a visual acuity of 20/100, while the second has similar cutaneous pigmentation and visual acuity of 20/20 and had not previously been recognized as having oculocutaneous albinism. Both brothers have foveal hypoplasia and misrouting of the optic fibers at the chiasm. Biochemical analysis suggests that this is a tyrosinase-related type of oculocutaneous albinism. This study demonstrates that careful observation of foveal development in relatives with normal vision is necessary to detect all individuals with albinism in a family. A suspected diagnosis of albinism may be confirmed when the visual-evoked potentials show excessive decussation of the optic fibers at the chiasm.

10. Other

- Dixon DM, Polak-Wyss A.
The medically important dematiaceous fungi and their identification. Mycoses 34:1-18, 1991.
Abstract : Dematiaceous fungi include a large group of organisms that are darkly pigmented (dark brown, olivaceous, or black). In most cases the pigment is melanin, and specifically, dihydroxynaphthalene melanin. The diseases produced include chromoblastomycosis, eumycotic mycetoma, and phaeohyphomycosis. Phaeohyphomycosis is a new classification for a diverse group of previously known entities grouped together on the basis of finding dematiaceous hyphal and/or yeast-like forms in tissue; tissue involvement may be superficial, cutaneous and corneal, subcutaneous, or systemic. Identification of these fungi is based mostly upon morphology. Important structures include annellides (Phaeoannellomyces, Exophiala), phialides (Phialophora, Wangiella), adelophialides (Phialemonium without collarettes, Lecytophthora with collarettes), differentiation of conidiophores (Xylohypha versus Cladosporium) and conidial hilum, septation and germination (Bipolaris, Drechslera, Exserohilum). Useful laboratory tests include the 12% gelatin test (controversial), nitrate assimilation (W. dermatitidis is negative, most other species are positive), and determination of temperature maxima (especially 37 degrees C for E. jeanselmei, 40 degrees C for

W. dermatitidis and B. spicifera, 42 degrees C for X. bantiana, and 45 degrees C for Dactylaria constricta var. gallopava and Scedosporium inflatum).

- Shiono H, Akane A.

A review of the sex determination of forensic specimens by DNA analysis. Application of the polymerase chain reaction (PCR) technique. Saishin Igaku 46:2278-2289, 1991.

Abstract : A review, with 32 refs., on the sex detn. of forensic samples using PCR amplifying DYZ 1 repetitive sequence on Y chromosome and amelogenin gene on X and Y chromosome. Amplification of the gene gives different length products between X and Y chromosome. DYZ 1 amplification can be carried out with 0.24 ng DNA sample, and shows higher sensitivity than the amelogenin amplification method. Amelogenin amplification requires 250 ng DNA samples, and have benefit to det. whether the anal. succeeded or failed. The method cannot be applicable to a single hair sample, partly due to inhibition of Taq polymerase by melanin.

- Zhdanova NN, Vasilevskaia AI, Artyshkova LV, Gavriliuk VI, Lashko TN, Sadovnikov I.

Complexes of soil micromycetes in the area of the influence of the Chernobyl Atomic Electric Power Station. Mikrobiol Zh 53:3-9, 1991.

Abstract : Complexes of soil micromycetes in the Chernobyl 30-km zone of the Ukrainian Polesye were studied for 1986-1989 with regard for such ecological parameters as the level of radiation contamination, a particular observation site, depth of soil horizon and season. As a result of the study correlation pleiads of soil micromycete complexes have been revealed with their structure and fungal genera characteristic of such complexes determined. The overwhelming majority of correlation pleiads of fungal complexes are attributed to complex-organized ones and this indicated high radioresistance of mycobiota in the studied, soils. Melanine-containing genera of fungi rank among the first in formation of correlation pleiads of soil micromycete complexes.

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The Pan American Society for Pigment Cell Research
IVth Annual Meeting
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IVth Annual Meeting of the European Society for
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