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RESEARCH

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Dear Member,

You should already have received the first issue of the Bulletin. Many members have encouraged me to continue, and that is the reason for my letter to you, after I had talked to G. Prota and O. Riley.

I would appreciate it very much if you would consider contributing to future issues by sending me a short text of 1 or 2 pages of either commentaries, or critical discussions, or critical reviews, or letters to the editor, on a subject related to your work on pigment cells.

Contributions are badly needed if we are to stimulate our members with new ideas and critical analyses in our Bulletin. This kind of short contribution has no place in a regular scientific journal such as the new "Pigment Cell Research" edited by Joe Bagnara, and to which, I hope, you will also send manuscripts. Please let me know at your earliest convenience if you are to contribute to the "Pigment Cell Bulletin". For your convenience, I enclose a form that you are kindly asked to fill in and send back to me.

I look forward to hearing from you. Yours sincerely,

Ferdy J. Lejeune, Editor

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**PIGMENT CELL RESEARCH BULLETIN**

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I shall send my contribution in the form of :

- COMMENTARY  \*
- DISCUSSION
- REVIEW
- LETTER TO THE EDITOR

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TOPIC COVERED : .....  
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# LETTER TO THE EDITOR OF



## PIGMENT CELL BULLETIN

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Dear Prof. Lejeune,

I would like to congratulate you on the appearance of the first number of the ESPCR Bulletin, which promises to be a most useful publication. I think that the literature survey is most helpful, and the Bulletin is also naturally a good vehicle for official communications. However, I am sure that one of its most important roles will be for the exchange of "News and Views". I am sure that you and your Editorial Board will wish to encourage members of the ESPCR to air their views informally about pigment cell biology and the affairs of the Society.

With regard to the latter, I believe that there are some areas that urgently need to be discussed in public. One of these concerns the question of the membership of Eastern European Colleagues. Many of our colleagues in Eastern Europe face problems of paying the subscription to the Society because of official restrictions on the exchange of currency. When the Council of the ESPCR discussed this problem last year, it was decided that it would be iniquitous to introduce a two-tier system of membership to the Society, and some arrangements would have to be made to enable Eastern European Members to pay their subscriptions. The solution to this appears to be to set up local societies in each of the Eastern Europe countries affected by the currency restrictions, so that these societies could receive the subscriptions from active members, and that at intervals, the monies accruing to these societies could be spent on the organization of Scientific meetings of the ESPCR in those countries.

Another matter of concern is the subscription to the journal "Pigment Cell Research". As you will have seen from the previous issue of the Bulletin, the journal is edited by Prof. J. Bagnara, and is



to be published by Alan Liss Inc. The present indications are that the amount of the annual subscription will be in the range of \$40 per annum for the first three years, assuming a mandatory membership subscription from members of the ESPCR. At the present rate of exchange, this would essentially double the annual subscription to the Society if the journal subscription were consolidated into the annual subscription for ESPCR members.

There will be an opportunity at the forthcoming General Assembly of the Society, which will be held during the Sorrento meeting in October, for members to express their views, but it is often valuable to have a public debate since frequently the free exchange of opinions results in constructive solutions to problems being proposed.

Yours sincerely,

P.A. RILEY  
Hon. Secretary ESPCR

# CURRENT LITERATURE IN



We acknowledge the valuable assistance of Ms Linda ALBRECHT and the financial support of Lawrence M. Gelb Research Foundation.

## PIGMENT CELL RESEARCH

### 1. MSH, OTHER HORMONES, DIFFERENTIATION

- Andersen AC, Jegou S, Eberle AN, Tonon MC, Pelletier G, Vaudry H  
Coexistence of melanin-concentrating hormone (MCH) and alpha-melanocyte-stimulating hormone (alpha-MSH) in the preoptic nucleus of the frog brain. *Brain Res Bull* 18:257-259, 1987.

Abstract : Coexistence of MCH- and alpha-MSH-like peptides in specific neurons of the frog hypothalamus has been investigated on serial frozen sections using the indirect immunofluorescence method. In the anterior region of the preoptic nucleus, perikarya containing MCH- and alpha-MSH-immunoreactive materials were co-distributed and the two peptides were generally co-sequestered within the same neurons. In contrast, alpha-MSH immunoreactive neurons of the ventral infundibular nucleus did not contain any MCH-like peptide. These data suggest that MCH and alpha-MSH are transported by the same nerve fibers originating from preoptic perikarya and are likely released together by axon terminals. Since MCH and alpha-MSH exert antagonistic hormonal activities on dermal melanophores, our results suggest that the two regulatory peptides may also interact in the central nervous system.

- Barber LD, Baker BI, Penny JC, Eberle AN  
Melanin concentrating hormone inhibits the release of alpha-MSH from teleost pituitary glands. *Gen Comp Endocrinol* 65:79-86, 1987.  
Abstract : A radioimmunoassay was developed for salmonid melanin concentrating hormone (MCH) and used to measure immunoreactive (ir)MCH in the hypothalamus and pituitary of trout (*Salmo gairdneri*) and eels, (*Anguilla anguilla*) maintained under different regimes of background colour. In trout, 95% of the total irMCH was located in the pituitary gland. The amount of MCH in both pituitary and hypothalamus was increased when white-adapted trout were transferred to a black background. In eels, a similar change of background led to an accumulation of MCH in the pituitary but not in the hypothalamus. The results suggest that MCH is released from the neurohypophysis in association with physiological color change. Neurointermediate lobes of trout and eels released both ir alpha-MSH and irMCH when they were cultured in vitro. The release of alpha-MSH was significantly enhanced when endogenous MCH was immunoabsorbed by MCH antiserum added to the culture medium. The results indicate that MCH can induce pallor in fish not only by its peripheral effect on the melanophores but also by an inhibitory action on the release of alpha-MSH from the pituitary.

- Castrucci AM, Hadley ME, Wilkes BC, Zechel C, Hruby VJ  
Melanin concentrating hormone exhibits both MSH and MCH activities

on individual melanophores. Life Sci 40:1845-1851, 1987.

Abstract : Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val (melanin concentrating hormone, MCH) and several fragment analogs (MCH1-14, MCH5-17, MCH5-14) were synthesized and their biological activities determined in a very sensitive fish skin bioassay. The potency ranking and minimum effective doses of the peptides were determined to be : MCH1-17 (10<sup>-12</sup>M) greater than less than MCH5-17(10<sup>-12</sup>M) greater than MCH1-14 (10<sup>-11</sup>M) greater than MCH5-14 (2 x 10<sup>-10</sup>M). The melanosome aggregating activity of MCH could be completely reversed by a 100-fold higher concentration of pounds-MSH. MCH was self-antagonized in a dose-related manner by higher concentrations of the peptide as was the activity of the MCH1-14 fragment analog. The MCH activities of the MCH5-17 and MCH5-14 analogs were not compromised by even the highest concentrations of the peptides employed. The MSH-like activity of MCH appears to relate to the N-terminus of the peptide whereas MCH activity is more a function of the C-terminus of the hormone. Self-antagonism of MCH at high concentrations appears to relate to the N-terminal tetrapeptide, which is responsible for the intrinsic MSH-like activity of the hormone.

- Hadley ME, Wood SH, Lemus-Wilson AM, Dawson BV, Levine N, Dorr RT, Hruby VJ

Topical application of a melanotropic peptide induces systemic follicular melanogenesis. Life Sci 40:1889-1895, 1987.

Abstract : We determined the relative effectiveness of alpha-MSH and a highly potent melanotropin analogue, (Nle<sup>4</sup>, D-Phe) - alpha-MSH, in stimulating a shift from pheomelanogenesis to eumelanogenesis within hair bulbs of mice. The analogue proved to be at least a hundred times more effective than the native hormone when injected subcutaneously. The two melanotropins were then incorporated into an ointment base and topically applied to a shaved area of the skin on the back of a yellow strain of mice (C57BL/6JAY). Within 24-48 hours eumelanin production was visible within hair bulb melanocytes in both treated and untreated areas of animals. The presence of melanized organelles (eumelanosomes) within melanocytes was confirmed by electron microscopy. These results document the delivery of a peptide hormone through the skin and into the systemic circulation. This is the first demonstration of the delivery of a peptide hormone by percutaneous absorption and may provide a model for a similar route of delivery of other peptide hormones. The hormone analogue has also been delivered across human skin in vitro. Delivery of a melanotropin by a transdermal route may prove to be clinically useful in the treatment of some integumental hypopigmentary disorders in humans.

## **2. MORPHOLOGY OF PIGMENT CELLS AND PIGMENTARY DISORDERS**

- Boissy RE, Moellmann GE, Lerner AB

Morphology of melanocytes in hair bulbs and eyes of vitiligo mice. Am J Pathol 127:380-388, 1987.

- Bruner JM

Tumors of Schwann's cells and pigmented skin cells. Clin Lab Med 7:181-198, 1987.

Abstract : Both melanocytes and peripheral nerve sheath cells have an established origin from the neural crest. Benign and malignant neoplasms of these cells have distinctive ultrastructural features, but there is a group of tumors that has some characteristics of both cell types. The distinguishing feature of melanocytic neoplasms is the presence of cytoplasmic premelanosomes, which indicates cellular synthesis of melanin. Nerve-sheath neoplasms may show ultrastructural variation, but are generally found to have long cell processes associated with basal lamina-like material. Immunocytochemistry can be used to help distinguish tumors of melanocytic and Schwann's cells from unrelated neoplasms.

- Cole GW, Barr RJ

Hypomelanosis associated with a colonic abnormality. A possible result of defective development of the neural crest. Am J Dermatopathol 9:45-50, 1987.

Abstract : A neonate born of black parents displayed a congenital, dramatic deficiency of most of his normal pigmentation. This was accompanied by a markedly dilated colon and various other defects. Light- and electron-microscopic examination revealed a deficiency in melanin content in the hypopigmented skin as compared to the normally pigmented areas. No other defects were noted. The possibility exists that a single aberration in neural crest development, a neurocristopathy, might be responsible for our patient's multiple congenital defects. Similar conditions in veterinary medicine and human disease reviewed and compared to this case.

- David M, Shanon A, Hazaz B, Sandbank M

Diffuse, progressive hyperpigmentation : an usual skin manifestation of mycosis fungoides. J Am Acad Dermatol 16:257-260, 1987.

- DiCostanzo DP, Urmacher C

Primary malignant melanoma of the esophagus. Am J Surg Pathol 11:46-52, 1987.

- Falabella R, Escobar C, Giraldo N, Rovetto P, Gil J, Barona MI, Acosta F, Alzate A.

On the pathogenesis of idiopathic guttate hypomelanosis. J Am Acad Dermatol 16:35-44, 1987.

- Kint A, Oomen C, Geerts ML, Breuillard F.

Congenital diffuse melanosis. Ann Dermatol Venereol 114:11-16, 1987.

### **3. MELANIN CHEMISTRY, BIOLOGY, HISTOCHEMISTRY AND OTHER PIGMENTS**

- Brewer NR

Comparative metabolism of copper. J Am Vet Med Assoc 190:654-658, 1987.

Abstract : Copper is required in trace amounts for many body functions. The prominent effects of Cu deficiency or Cu toxicosis differs greatly between animal species. Along with iron, Cu is necessary for the transfer of O<sub>2</sub> via a cascade of enzymes so that energy may be available for vital body functions without overheating of the tissues through rapid oxidation. As a part of lysyl oxidase, Cu has an obligate function in the maturation of all connective tissue (including elastic tissue and bone) maintaining the form and integrity of all body organs. As a constituent of tyrosinase, Cu is involved in the formation of melanin, thus preventing albinism. Copper also is involved in the myelination of nerve fibers and the production of neutrophils, enkephalins, lipoproteins and cholesterol. Copper must be properly sequestered to prevent toxicosis. Copper is stored primarily as metallothioneins and as superoxide dismutase and is transported primarily as ceruloplasmin or as low molecular weight proteins, peptides and amino acids.

- Cabanes J, Garcia-Canovas F, Lozano JA, Garcia-Carmona F.  
A kinetic study of the melanization pathway between L-tyrosine and dopachrome. *Biochim Biophys Acta* 923:187-195, 1987.

Abstract : In the pathway of melanin biosynthesis originating from L-tyrosine, the dopachrome accumulation at physiological pH is produced with a pronounced lag period, during which the level of L-dopa increases, following a sigmoidal kinetics to reach a steady-state. A kinetic model has been proposed for the overall pathway of melanization from L-tyrosine to dopachrome; it explains the lag period present during the dopachrome accumulation as well as the influence of L-tyrosine and tyrosinase over this lag period. Use of this model is also valid to explain the kinetics of L-dopa accumulation in the reaction medium, as has been tested by simulation.

- Charman WN, Farquhar CJ, Finnin BC, Reed BL  
Characterization of biopolymers by pyrolysis gas chromatography and multidimensional analysis. Application to synthetic melanins. *J Chromatogr* 388:389-396, 1987.

- Chedekel MR, Subbarao KV, Bhan P, Schultz TM  
Biosynthetic and structural studies on pheomelanin. *Biochim Biophys Acta* 912:239-243, 1987.

Abstract : <sup>13</sup>C-NMR spectroscopy of pheomelanin biopolymers, prepared from isotopically enriched precursors, has been developed as a tool for structure elucidation of melanins. By employing large pulse-widths and short cycle time, only the signals originating from labeled carbons are observed in the high-resolution spectra of these polymers.

- Muske LE, Fernald RD  
Control of a teleost social signal. I. Neural basis for differential expression of a color pattern. *J Comp Physiol* 160:89-97, 1987.

#### **4. NEUROMELANINS**

- Bianchi M, Merighi A.

The relationship between lipofuscin and neuromelanin in some sites of the nervous system of the horse. *Exp Biol* 46:89-99, 1986.

- D'Amato RJ, Alexander GM, Schwartzman RJ, Kitt CA, Price DL, Snyder SH

Neuromelanin : a role in MPTP-induced neurotoxicity. *Life Sci* 40:705-712, 1987.

**Abstract** : Methylphenyltetrahydropyridine (MPTP) selectively destroys melanin-containing neurons in the substantia nigra of humans and other primates. Methylphenylpyridine (MPP+), an active metabolite of MPTP, which is accumulated intraneuronally by the catecholamine uptake system, binds with high affinity to neuromelanin. MPP+ bound intracellularly to neuromelanin may be released gradually, resulting in damage to the neurons of the substantia nigra. Chloroquine, a drug which blocks MPP+ binding to neuromelanin, can protect monkeys from MPTP neurotoxicity.

- D'Amato RJ, Benham DF, Snyder SH

Characterization of the binding of N-methyl-4-phenylpyridine, the toxic metabolite of the parkinsonian neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, to neuromelanin.

- Damron TA, Schelper RL, Sorensen L.

Cytochemical demonstration of neuromelanin in black pigmented adrenal nodules. *Am J Clin Pathol* 87:334-341, 1987.

- Erwin WG, Turco TF

Current concepts in clinical therapeutics : Parkinson's disease. *Clin Pharm* 5:742-753, 1986.

- Grisham MB, Perez VJ, Everse J.

Neuromelanogenic and cytotoxic properties of canine brainstem peroxidase. *J Neurochem* 48:876-882, 1987.

- Wu EY, Chiba K, Trevor AJ, Castagnoli N Jr.

Interactions of the 1-methyl-4-phenyl-2,3-dihydropyridinium species with synthetic dopamine-melanin. *Life Sci* 39:1695-1700, 1986.

#### **5. PHOTOBIOLOGY AND PHOTOCHEMISTRY**

- Holick MF

Photosynthesis of vitamin D in the skin : effect of environmental and life-style variables. *Fed Proc* 46:1876-1882, 1987.

**Abstract** : Exposure to sunlight continues to play a major role in providing adequate vitamin D nutrition for most of the population of the world, including those who live in countries that practice fortification of dairy, margarine, and cereal products with vitamin D. During exposure to sunlight, the high-energy UV photons (290-315 nm) penetrate the epidermis and photolyze 7-dehydrocholesterol (provitamin D<sub>3</sub>) to previtamin D<sub>3</sub>. Once formed, previtamin D<sub>3</sub>

undergoes a thermally induced isomerization to vitamin D3 that takes 2-3 days to reach completion. Melanin effectively competes with provitamin D3 for the UV radiation that enters the epidermis and limits its photolysis to previtamin D3. However, this is not the major factor that prevents excess production of vitamin D in the skin of people who are constantly exposed to sunlight. During the initial exposure to sunlight, provitamin D3 is efficiently converted to previtamin D3. However, because previtamin D3 is photolabile, continued exposure to sunlight causes the isomerization of previtamin D3, principally to lumisterol. Thus, no more than 10-20% of the initial provitamin D3 concentrations ultimately end up as previtamin D3. Aging, sunscreens, seasonal changes, time of day, and latitude also significantly affect the cutaneous production of this vitamin-hormone.

- Hotta H, To H.

Cosmetics containing 4-hydroxycinnamic acid and cyclodextrin for the prevention of melanin formation. Jpn Kokai Tokyo Koho JP 62/19510 A2 (87/19510), 1987.

- Joshi PC, Carraro C, Pathak MA

Involvement of reactive oxygen species in the oxidation of tyrosine and dopa to melanin and in skin tanning. Biochem Biophys Res Commun 142:265-274, 1987.

Abstract : The role of reactive oxygen ( $^1O_2$  and  $O_2^-$ ) in skin photosensitization and tanning reaction has been examined. Riboflavin (RF), hematoporphyrin (HP), 3-carbethoxyypsoralen (3-CP), and 8-methoxyypsoralen (8-MOP), upon photoexcitation under aerobic conditions, produced singlet  $O_2$  ( $^1O_2$ ). RF, 3-CP, and 8-MOP also produced superoxide anion ( $O_2^-$ ). Reactive  $O_2$  produced by photosensitized RF, 3-CP, and 8-MOP was found to oxidize tyrosine and dopa to dopachrome and subsequently their conversion to melanin. HP did not oxidize tyrosine to dopachrome, and 3-CP and RF revealed substantial oxidation of tyrosine. Dopa was oxidized to dopachrome and subsequently to melanin by all photosensitizers tested at a variable rate as follows : RF greater than 3-CP greater than HPD greater than 8-MOP. UVA alone and to a lesser extent UVB also produced  $^1O_2$  which induced the oxidation of tyrosine and dopa to dopachrome and subsequently to melanin. The production of dopachrome was higher with dopa compared to tyrosine under all irradiation conditions. These observations appear to have relevance to the  $O_2^-$ -requiring immediate tanning reaction of the skin stimulated by solar radiation and in the induction of skin photosensitization.

- Korytowski W, Pilas B, Sarna T, Kalyanaraman B

Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanins. Photochem Photobiol 45:185-190, 1987.

## **6. MELANOMA**

- Houghton AN, Real FX, Davis LJ, Cordon-Cardo C, Old LJ

Phenotypic heterogeneity of melanoma. Relation to the differentiation program of melanoma cells. J Exp Med 165:812-829, 1987.

Abstract : Phenotypic heterogeneity is a characteristic feature of tumor lesions in patients with melanoma. Variability can be observed in cell morphology, pigmentation, and antigen expression. To test whether phenotypic heterogeneity could be the result of events regulated during cell differentiation, we evaluated the expression of a panel of differentiation traits on melanoma cells. Metastatic melanoma lesions from two patients, designated FD and AP, were examined histologically and found to contain mixed populations of cells. Established melanoma cell lines derived from each of these lesions were subcloned at early passage in culture (passages heterogeneity in the expression of differentiation-related traits in clones, corresponding to distinct phenotypes observed within the original tumors. Clones from patient FD corresponded to early to intermediate stages of melanocyte differentiation, and clones from patient AP ranged from intermediate to late stages. The influence of cholera toxin and PMA on differentiation of parental cultures and subclone was studied. Results of induction studies demonstrated a number of features of differentiation of melanoma cells : regulation of differentiation traits is coordinated as a program of traits expressed sequentially at specific stages; early traits,, such as the epidermal growth factor receptor and the melanoma chondroitin sulfate proteoglycan antigen, are downregulated as melanoma cells differentiate, whereas late markers, including melanin, tyrosinase activity, and antigens expressed in mature melanosomes, are upregulated; Ia (class II major histocompatibility) antigens are characteristically expressed on melanomas corresponding to early or intermediate stages of differentiation and are regulated as part of the differentiation program; minimal changes in stage of differentiation were observed during induction of parental cultures with either cholera toxin or PMA, whereas definite shifts in differentiation could be induced in selected cloned subpopulations. We conclude that melanoma cells are not frozen at a specific stage of differentiation, but rather are capable of differentiating when exposed to appropriate signals. Diversity in the differentiation state of melanoma cells can account for much of the phenotypic heterogeneity observed in melanoma lesions.

- Johnson CW, Barth RF, Adams D, Holman B, Price JE, Sautins I  
Phenotypic diversity of murine B16 melanoma detected by anti-B16 monoclonal antibodies. Cancer Res 47:1111-1117, 1987.

- Ralfkiaer E, Hou-Jensen K, Gatter KC, Drzewiecki KT, Mason DY  
Immunohistological analysis of the lymphoid infiltrate in cutaneous malignant melanomas. Virchows Arch 410:355-361, 1987.

Abstract : The immunological phenotypes of the lymphoid cells in 39 cutaneous malignant melanomas have been investigated by staining cryostat sections with a panel of 20 monoclonal antibodies against lymphoid cells and their subsets. Staining was performed by the alkaline phosphatase : anti-alkaline phosphatase (APAAP) method in which the substrate label (red) is easily distinguishable from melanin. The lymphoid infiltrates had an essentially identical composition in all cases, consisting of T-lymphocytes associated with both Langerhans cells and HLA-DR-positive tissue macrophages. B-lymphocytes and natural killer cells were either absent or only



present in low numbers. The ratio between T8 (suppressor/cytotoxic) and T4 (helper/inducer) lymphocytes varied and showed no correlation with melanoma subtype, level of invasion or magnitude of lymphocytic response. Examination for markers associated with T-cell activation and/or with cell proliferation revealed that all lesions contained HLA-DR-positive T-lymphocytes, whereas expression of the transferrin receptor and the interleukin-2 receptor (Tac-antigen) occurred mainly in melanomas with a significant inflammatory infiltrate. These data support the concept that malignant melanomas are capable of evoking autologous T-cell immune reactions.

- Traboulsi EI, Jalkh AE, Frangieh GT, Tomb J  
Vitreous histocytology of primary choroidal malignant melanoma. Ann Ophthalmol 19:45-47, 1987.

- Winston KR, Sotrel A, Schnitt SJ  
Meningeal melanocytoma. Case report and review of the clinical and histological features. J Neurosurg 66:50:-57, 1987.

## **7. TYROSINE AND OTHER ENZYMES**

- Burchill SA, Thody AJ  
Melanocyte-stimulating hormone and the regulation of tyrosinase activity in hair follicular melanocytes of the mouse. J Endocrinol 111:225-232, 1986.

**Abstract** : Skin tyrosinase activity increases during hair growth in C3H-HeAvy mice and reaches higher levels in young (30- to 35-day old) mice when the hair follicular melanocytes synthesize the black pigment, eumelanin, than in older (6-month old) mice when they produce the golden yellow pigment, pheomelanin. To examine the regulation of the melanocytes at these different stages we have compared the effect of alpha-MSH and other agents that act, through cyclic AMP-dependent mechanisms, on skin tyrosinase activity in both young and old mice during hair growth, initiated by plucking. Daily administration of alpha-MSH, isoprenaline or theophylline increased coat darkness, and skin tyrosinase activity in the younger mice 7-9 days after plucking, but they were ineffective in the older mice. Similarly alpha-MSH, 8-bromo-cyclic AMP or theophylline increased tyrosinase activity in skin explants from the younger mice incubated for up to 24 h but had no effect in explants from older mice. Cyclic GMP had no effect on tyrosinase activity in skin explants from both young and old mice. It is suggested that whereas cyclic AMP-dependent mechanisms may operate to regulate tyrosinase activity in the hair follicular melanocytes of younger mice that produce eumelanin these systems may not operate in the older mice when these melanocytes synthesize pheomelanin. Pheomelanin synthesis, unlike that of eumelanin, may not depend upon tyrosinase and its regulation by cyclic AMP and this could explain the two levels of this enzyme in the skin and its failure to respond to alpha-MSH and other activators of the cyclic AMP system during periods of pheomelanin production.

- Laskin JD, Piccinini LA

Tyrosinase isozyme heterogeneity in differentiating B16/C3 melanoma. *J Biol Chem* 261:16226-16235, 1986.

**Abstract** : The B16/C3 murine melanoma is a pigmented tumor that is rich in the copper-containing enzyme, tyrosinase. This enzyme, which converts tyrosine to melanin precursors, is largely associated with membrane fractions of cells and exists in a number of discrete isozymic forms ranging in molecular mass from 58,000 to 150,000 daltons and pI from 3.4 to 5.2. One of these isozymes (Mr = 58,000, pI 3.4) has been purified to homogeneity. The purified enzyme catalyzes the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and the conversion of L-DOPA to dopaquinone. Ascorbic acid, tetrahydrofolate, and dopamine can serve as cofactors in the hydroxylase reaction. The Michaelis constants for the purified enzyme were  $7 \times 10^{-4}$  M for L-tyrosine and  $6 \times 10^{-4}$  M for L-DOPA. The Vmax for L-DOPA was much greater than the Vmax for L-tyrosine indicating that tyrosine hydroxylation is rate-limiting in melanin precursor biosynthesis. Two putative copper chelators, phenylthiourea and diethyldithiocarbamide inhibited both the tyrosine hydroxylase and L-DOPA oxidase activities of the enzyme. Phenylthiourea was a noncompetitive inhibitor while diethyldithiocarbamide was a competitive inhibitor indicating that these agents act by different mechanisms. When digested with proteases and glycosidases, higher molecular weight forms of tyrosinase comigrated with the purified enzyme in isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis suggesting that the isozyme was derived from larger precursors. Thus, post-translational processing of tyrosinase may underlie isozyme diversity and this may be important in the control of melanogenesis in this tumor model.

- Martinez JH, Solano F, Arocas A, Garcia-Borron JC, Iborra JL, Lozano JA

The existence of apotyrosinase in the cytosol of Harding-Passey mouse melanoma melanocytes and characteristics of enzyme reconstitution by Cu(II). *Biochim Biophys Acta* 923:413-420, 1987.

## **8. EYE**

- Kemali M, Kemali D, Maj M, Lovero N, Milici N.

Frog retinal pigment screening and lithium. *Comp Biochem Physiol, C: Comp Pharmacol Toxicol* 860:421-423, 1987.

- Stroeva OG, Bibikova AD

Hormone-sensitive stage in the development of retinal pigment epithelium in genotypically normal rats. *Dokl Akad Nauk SSSR* 292:1249-1252, 1987.

# ANNOUNCEMENTS



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**RESEARCH OPPORTUNITY : POSTDOCTORAL SCIENTIST**

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL  
UNIVERSITY OF LONDON  
Department of Anatomy

Required for a 3-year project supported by the Wellcome trust, to work on the cellular and molecular biology of mouse coat-colour mutations which act in development. The research is aimed at the identification and cloning of a mammalian gene involved in the intracellular control of cell differentiation.

The position is in the group of Dr Dorothy Bennett, which has general interests in cell differentiation and cancer, especially melanoma. The Anatomy Department has strong research interests in vertebrate development and differentiation, and is well-equipped, spacious and friendly.

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