PIGMENT CELL RESEARCH BULLETIN

PUBLISHED BY THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

N° 10 - August 1990

Editor: Prof. F. Serri, Foundation for research in Dermatology, Catholic University, Largo A. Gemelli 8, 00168 Rome, Italy. Phone: 06-3385451. Fax: 06-3051343.
Editorial Office: Dr G. Ghanem (Assist. Edt), C. Henrotte (Production assist.) Lab. of Oncology and Exp. Surgery, Institut J. Bordet, 1, Rue Heger-Bordet, 1000, Brussels, Belgium. Phone: 32.2.539 23 43. Fax: 32.2.537 47 34.

CONTENTS

Periodico quadrimestrale della European Society for Pigment Cell Research (Associazione Europea per la Ricerca sulla Cellula Pigmentaria), realizzatto con il contributo della Fondazione Pro Ricerca Dermatologica e della Pfizer Italiana
Direttore Responsabile: Prof. Giuseppe Prota, Presidente ESPCR, Dip. di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, 80134 Napoli. Autorizzazione del Tribunale di Napoli n. 3684 dell'11/12/87.

LETTER TO THE EDITOR OF

PIGMENT CELL BULLETIN

REVIEW

Targeted radiotherapy for malignant melanomas

Eva M.Link
Department of Chemical Pathology, Academic Unit, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, U.K.

An exceptional malignancy of melanoma often manifested by a wide metastatic dissemination almost concomitant with the appearence of the primary tumour together with a notorious resistance of this neoplasm to all presently available therapeutic modalities justifies a search for new methods which would effectively assist both diagnosis and treatment of primary tumours and metastases even at very early stages of their development.

An availability of a wide range of radioisotopes initiated a new era called Nuclear Medicine in which an *in vivo* tracing of tissues of interest (either normal or malignant) became possible providing that a selective uptake of the appropriate radioisotope can be achieved. The development of diagnostic procedures then led to targeted radiotherapy. The latter could be defined as a radiotherapy selectively directed at a particular tissue, most often neoplastic, by using either a radioisotope exhibiting a high affinity to this tissue or a compound with such affinity employed as a carrier for a suitable radionuclide. The selectiveness of the radioisotope uptake enables deposition of high radiation doses in the targeted tissue with only minor exposure of remaining structures, unlike exposure associated with the classical beam radiotherapy in which both treated and adjacent tissues are irradiated nonspecifically.

The selectivity is the main problem in targeted radiotherapy since neither the already investigated carriers for radioisotopes nor radionuclides themselves exhibit exclusive affinity for malignant tissue. However, it should be emphasized that successful targeted radiotherapy depends not only on a proper selection of a carrier for radioisotopes. A judicious choice of the radionuclide is equally important: a selective uptake of radioisotopes in the targeted tissue has to be coupled with their appropriate physical properties conditional for the most effective treatment as follows:

1. high linear energy transfer (LET) of radiation emitted to achieve the highest density of ionization in the target on which the cytocidal effects are

dependent,

2. short range of emitted radiation to deposit most or even all radiation in a tumour without damaging its surrounding normal tissues,

3. relatively short half-life to minimize radiation doses to the normal organs in which the radioisotope can be accumulated if the carrier lacks an exclusive uptake limited to the targeted lesion(s),

4. safe daughter element to which the radionuclide decays.

(For the complete review of suitable radioisotopes for targeted radiotherapy see [1].)

TARGETING MELANOMA: RADIOISOTOPES

Two radioisotopes: phosphorus-32 (^{32}P) and gallium-67 (^{67}Ga) were of a particular interest as potential diagnostic radionuclides for melanomas. ^{32}P, a β^--emitter used mainly for detecting choroidal melanomas, was abandoned due to its insufficient specificity for the tumour and, additionally, to a poor tissue penetration of β-radiation emitted (approx.2mm) which resulted in the requirement of a surgical removal of the eye which enabled the ^{32}P uptake test [2].

^{67}Ga, exhibiting a relatively high affinity to several malignant lesions including melanomas, was employed to monitor metastatic dissemination for quite a long time. However, a recent retrospective review of the results obtained from more than 200 patients over 8 years indicated a limited value of the routine screening of melanoma patients with ^{67}Ga since the informations obtained were not superior to that derived from other conventional diagnostic procedures [3].

TARGETING MELANOMA: CARRIERS FOR RADIOISOTOPES

Since none of therapeutically useful radionuclides exhibits a specific affinity for melanomas, the use of an appropriate carrier is indispensable. Three classes of such carriers can be distinguished between those already investigated:
1. compounds with a high affinity to melanin;
2. compounds which can serve as a melanin precursor;
3. antibodies and, more recently, monoclonal antibodies.

1. Compounds with a high affinity to melanin

Several compouds exhibit an ability for binding to melanin with a variable degree of affinity to this bio-polymer. Between those already investigated are cocaine [4], kanamycin [5], streptomycin group of antibiotics [6], rifampicin [7], quinine [5], haloperidol [8], as well as chloroquine [5,9,10] and phenothiazine derivatives including chlorpromazine and methylene blue [11,12]. Some of these compounds when routinely used in the clinical practice caused secondary effects such as hearing and sight disturbances or a sensation of imbalance, among others [13-15]. A frequent persistance of the symptoms after completion of treatment led to a discovery of the association between these effects and a prolonged drug-melanin binding. This, in turn, initiated investigations which revealed a significant uptake of compounds with a high affinity to melanin in pigmented melanomas, and resulted in the use of such compounds as carriers for radioisotopes in diagnosis and treatment of this neoplasm. Chloroquine and two phenothiazine derivatives: chlorpromazine and methylene blue were studied most extensively. A chloroquine analoge: 4-(3-dimethylamino propylamino)-7-iodoquinoline

known as NM-113 and labelled with either ^{125}I, ^{131}I or ^{123}I appeared to be very efficient in the detection of melanoma [16,17]. Unfortunately, a high level of this radiopharmaceutical observed in the eye uvea for almost 1 year after injection excluded a possibility of using it for therapeutic purposes [5]. Similarly, chlorpromazine which exhibits relatively high affinity to melanin (52% uptake by choroidal pigment in suspension [9]) proved to be accumulated very effectively in pigmented melanomas: its level in the tumour exceeded several times those observed in non-pigmented tissues [18]. However, a high uptake in eyes [18] and, particularly, in the neuromelanin-containing cells, as discovered by autoradiography of human brain sections [5] (rodents do not possess neuromelanin) in addition to well known side effects (extrapyramidal disturbances [19]), limited investigations concerning therapeutic efficacy of radiolabelled chlorpromazine for melanomas to in vitro systems and animal models.

Methylene blue, unlike chlorpromazine, is relatively harmless (1-4 mg i.v./kg body weight/day is well tolerated [20]) and characterised by a significantly higher affinity to melanin in comparison to chlorpromazine (87% uptake by choroidal pigment in suspension [9]). Its bio-distribution is similar to that of chlorpromazine [21], but lack of secondary effects at the doses needed for targeted radiotherapy stimulated the use of this compound as a carrier for five different radioisotopes, namely ^{35}S (β-emitter) [22], ^{125}I (Auger electron emitter) [23] and ^{211}At (α-particle emitter) [23-25], as well as ^{123}I (γ-emitter)[26] and ^{124}I (β^+-emitter) [26], for therapeutic and diagnostic purposes, respectively.

2. Compounds which can serve as a melanin precursor

Selective uptake of some thioamides (characterised by thioureylene structure) observed in pigmented melanomas is due to a mechanism different from that of polycyclic amines discussed above [27]. Unlike chloroquine or phenothiazine derivatives which bind to the preformed melanin, thioamides serve as so called false precursors of this pigment. The uptake of such compounds occurs exclusively in structures in which the active melanin synthesis takes place at the time of the compound administration and in a proportion to the rate of melanin synthesis. Thioamides are, therefore, preferentially in developing or growing pigmented tissues at the expense of the already formed organs.

Extensive whole-body autoradiographic studies concerning radiolabelled analogue of thiouracil - one of the most selective false precursor of melanin in vivo - confirmed its high and long-term uptake in pigmented melanomas (growing tissue) with its concomitant low level in maternal eyes. However, foetal eyes, the developing organs, were easily identified in autoradiograms [28].

An application of false melanin precursors as a carrier for radioisotopes in the diagnosis and treatment of ocular melanomas should be particularly advantageous. A highly efficient accumulation of [^{123}I]-5-iodo-2-thiouracil in the tumour but a residual incorporation by choroidal melanin results in a beneficial tumour/eye ratio of the radioisotope uptake [29].

Therapeutic effects of [^{35}S]-thiouracil for non-ocular animal melanoma were also investigated with a promising regression of the tumours [30]. However, doses of 10mCi and higher given over a short period (24h) required to obtain such effect in mice (corresponding to approx. 28Ci in man) seem

unacceptable in the clinic. Nevertheless, labelling this compound with a more suitable radioisotope should prove to be very useful for targeted radiotherapy of melanoma.

Similarly to thiouracil, phenylalanine serves as a melanin precursor. It was used especially as a carrier for boron-10 (^{10}B) in unconventional targeted radiotherapy - boron neutron capture therapy - by two independent groups of investigators in Japan and USA, with very promising early clinical results achieved by the former (for review see Pigment Cell Res., 2(4), 1989).

3. Monoclonal antibodies

The idea of employing antibodies against a tumour as a carrier for radioisotopes is quite old. As early as in 1953 first antibodies against a tumour were prepared to show their affinity to the neoplastic tissue after injection into a tumour-bearing host. One decade later (1965) a radioiodination including a paired- and triad-label techniques was developed which enabled localization of antitumour antibody, anti-normal tissue antibody and control globulin at the same time (for review see [31]). A discovery of monoclonal antibodies made this technique widely available. An oncofetal antigen, P97, to which monoclonal antibodies are directed, is strongly expressed in many melanomas and only weakly in normal adult tissue. Murine monoclonal antibodies to P97 were shown to be safe for humans in milligram doses. Used as a carrier of ^{131}I for diagnostic purposes they revealed 88% of metastatic melanoma lesions but with their size not smaller than 1.5 cm in diameter [32]. Since it was obvious that whole IgG molecules were strongly immunogenic and remained in blood for a prolonged time, Fab and $F(ab)_2$ fragments were tested as less antigenic and rapidly cleared from extracellular fluid compartment. Again, it was not possible to localise melanoma lesions smaller than 1.5 cm [32]. However, monoclonal antibodies against the high molecular weight antigen enabled detection of melanoma deposits with their diameter under 1 cm [33,34].

The therapeutic approach with radiolabelled monoclonal antibodies has so far been less promising since heterogeneity of antigen expression, particularly on melanoma lesions, and limited access of antibodies to less vascularised tumours result in the uptake of radioisotopes below the level required for the therapeutic effects to be achieved. Additionally, a poor penetration of antibodies within the neoplastic lesions forces a choice of radionuclides characterised by a longer range of emitted radiation which are consequently usually less therapeutically effective and carry a higher probability of damaging the tissue surrounding the tumour [34,35]. These problems need to be solved for radiolabelled monoclonal antibodies to be successfully applied in targeted radiotherapy for cancer including melanomas.

CURRENT STATUS

Most advanced investigations concern a clinical diagnosis of disseminated melanoma with radiolabelled monoclonal antibodies, boron neutron capture therapy of cutaneous, well localised lesions of this neoplasm using ^{10}B-phenylalanine, and targeted radiotherapy of disseminated melanoma with [^{211}At]-methylene blue.

At present, boron neutron capture therapy arouses the greatest interest since the first clinical treatment of a large, inoperable melanoma on the left occiput, with ^{10}B-para-boronophe-

nylalanine hydrochloride proved to be very successful [36]. Boron neutron capture therapy is a form of targeted radiotherapy in which non-radioactive isotope accumulated in a tumour due to its carrier is exposed to thermal neutrons. The activated ^{10}B decays to ^{7}Li in $^{10}B(n,\alpha)^{7}Li$ reaction emitting α-particles. This high LET radiation derived from ^{10}B selectively localised in the tumour exhibits almost maximal therapeutic efficacy and, being characterised by a short range of 10-14μm (a diameter of approx. 1 cell), does not damage normal tissues surrounding the neoplastic lesion. Up to date the neutron capture therapy is limited to superficial and well localised melanomas since neither the poor penetration of thermal neutrons used nor co-induction of τ-rays allow a whole-body irradiation. However, some progress is being made to overcome these difficulties [37].

Another radiolabelled compound for targeted radiotherapy which proved to be particularly suitable for disseminated melanoma is [^{211}At]-methylene blue [23-25]. [^{211}At]-methylene blue was selected from three radioanalogues of this compound, namely, β-emitting [^{35}S]-methylene blue [22], Auger electron-emitting [^{125}I]-methylene blue [23] and α-particle emitting [^{211}At]-methylene blue [23] since the therapeutic efficacy of the latter exceeded that found with the former two radioanalogues by two orders of magnitude. ^{211}At is an α-particle emitter; the features of its densely ionising radiation consist of the optimal LET of almost 100keV/μm, a short half-life of 7.2h and, similarly to activated ^{10}B, a short mean range of 60-65μm in tissue which corresponds to a diameter of 3-6 cells. [^{211}At]-methylene blue shows its exceptional effectiveness in scavenging single melanoma cells circulating with blood (as assessed by determination of a number and size of lung and lymph node metastases appearing after i.v. injection of human melanoma cell suspension [24] or lymph node metastases after subcutaneous implantation of human melanoma xenografts [25] into nude mice subsequently treated with [^{211}At]-methylene blue). This astatinated compound manifests its therapeutic properties also towards cutaneous lesions which, however, significantly dependent on the tumour size at the time of treatment [25]. Up to date the irreversible regression of cutaneous lesions with a concomitant growth inhibition of metastases in lymph nodes was observed for tumours with a mean diameter of 5-6 mm which, if the ratio of tumour mass to the body weight is taken into account, would correspond to the lesion of 1-2 cm in diameter in man [25]. Since doses needed to cause a complete regression of cutaneous and metastatic lesions, including those expected in normal pigmented organs, should be well tolerated by patients, the results achieved encouraged us to introduce the treatment to the clinical trials.

Summarising all pros and cons for boron neutron capture therapy and targeted radiotherapy with [^{211}At]-methylene blue, it seems that a combination of both methods: the former for well localized, even large tumours, and the latter for systemic treatment of disseminated lessions (often "preventive", without a possibility of diagnostic confirmation due to a microscopic size of the tumours) could result in a significant improvement of the prognosis for melanoma patients.

References

1. Humm JL: J Nucl Med, 27, 1490-1497, 1986
2. van Dijk RA: Doc Ophthalmol, 46, 1-32, 1978
3. Kagan R, Witt T, Bines S, Mesleh G, Economou S: Cancer, 61, 272-274, 1988
4. Patil PN: Invest Ophthalmol,

11, 739-746, 1972
5. Lindquist NG: Acta Radiol, Suppl. 325, 1-92, 1973
6. Dencker L, Lindquist NG, Ullberg S: Experientia, 29, 1362-1364,1973
7. Boman G: Acta Ophthalmol, 51, 367-370, 1973
8. Lyden A, Larsson B, Lindquist NG: Arch Inter Pharm Ther, 259, 230-243, 1982
9. Potts AM: Invest Ophthalmol, 3, 399-416, 1964
10. Mitchell W, Epstein JH: J Invest Dermat, 45, 482-488, 1965
11. Potts AM: Tr Am Ophthalmol Soc, 60, 517-552, 1962
12. Potts AM: Invest Ophthalmol, 1, 522-530, 1962
13. Goard EL, Fletcher MC: Tr Am Ophthalmol Soc, 54, 129-139, 1956
14. Arden GB, Friedmann A, Kolb H: Lancet, 1, 1164, 1962
15. Scherbel AL, Harrison JW, Atdjian M: Cleveland Clin Q, 25, 95, 1958
16. Boyd M, Lieberman LM, Reierwaltes WH, Varma VM: J Nucl Med, 11, 479-486, 1970
17. Packer S, Redvanly C, Lambrecht RM, Wolf AP, Atkins HL: Arch Ophthalmol, 93, 504-508, 1975
18. Blois MS: J Invest Derm, 45, 475-481, 1965
19. Ayd FJ: J Am Med Assoc, 175, 1054-1061, 1961
20. Martindale: The Extra Pharmacopoeia, (ed.A.Wade), 27th edition, The Pharmaceutical Press, London, pp.333-334, 1977
21. Link EM, Rydzy M: in: Proceedings of the Third Meeting of the Polish Biophys Soc, 62, 1978
22. Link EM, Lukiewicz S: Eur J Nucl Med, 7, 469-473, 1982
23. Link EM, Brown I, Carpenter RN, Mitchell JS: Cancer Res, 49, 4332-4337, 1989
24. Link EM, Carpenter RN: Cancer Res, 50, 2963-2967, 1990
25. Link EM, Carpenter RN, Hansen G: manuscript in preparation
26. Link EM, Spittle MF, Ell PJ, Jones T et al: current investigations
27. Olander K, Larsson B, Dencker L: Acta Pharmacol Toxicol, 52, 135-142, 1983
28. Dencker L, Larsson B, Olander K, Ullberg S, Yokota M: Br J Cancer, 39, 449-452, 1973
29. Franken NAP, van Langevelde A, Journee-de Korver JG, Bakker CNM, Feitsma RIJ, van Delft JL, Oosterhuis JA, Pauwels EKJ: Nucl Med Comm, 6, 657-663, 1985
30. Fairchild RG, Coderre JA, Packer S, Greenberg D, Laster BH: Int J Radiat Oncol Biol Phys, 17, 337-343, 1989
31. Pressman D: Cancer Res, 40, 2960-2964, 1980
32. Larson SM, Carrasquillo JA, Krohn KA, Brown JP et al: J Clin Invest, 72, 2101-2114, 1983
33. Siccardi AG, Buraggi GL, Callegaro L et al: Cancer Res, 46, 4817-4822, 1986
34. Larson M: Radiology, 165, 297-304, 1987
35. Kwok CS, Crivici A, MacGregor WD, Unger MW: Cancer Res, 49, 3276-3281, 1989
36. Mishima Y, Ichihashi M, Hatta S, Honda C, Yamamura K, Nakagawa T: Pigment Cell Res, 2, 226-234, 1989
37. Fairchild RG, Slatkin DN, Coderre JA et al: Pigment Cell Res, 2, 309-318, 1989.

****

REVIEW

Estrogens and growth of malignant melanoma

Myra K. Jacobsohn and Gert M. Jacobsohn
Department of Biological Chemistry, Hahnemann University, Philadelphia, PA 19102, USA

Melanin, the brown-black polymer in pigment cells, is being recognized increasingly as a non-specific binding agent for various organic substances. This binding is in lieu of, or in addition to, receptor protein binding which has a relatively higher specificity than the melanin. We have found that melanin is capable of binding estrogens; 2-hydroxyestradiol, a metabolite of estradiol produced in the liver, brain and uterus, binds to a somewhat higher extent than estradiol (Jacobsohn et al, 1988). Melanin which is being actively synthesized from dopa binds more estradiol than preformed melanin (Jacobsohn et al, 1988). The binding is not a unique phenomenon. A number of aromatic substances have the ability to bind to melanin and this phenomenon has been used to explain the localization of drugs at their site of action or the concentration of toxins in specific locales where they may exercise a damaging effect. The binding may also remove harmful substances from causing damage elsewhere. The successful treatment of tinnitus by lidocaine injection was thought by Lyttkens (1986) to be due to the drug accumulating on melanin of the inner ear. He found lidocaine, bupivacaine and chlorpromazine, a phenothiazine derivative, to associate with melanin-containing structures of the eye and of the inner ear (Lyttkens et al, 1979). Melanin of the eye, hair and skin of pigmented mice can take up serotoni, dopamine, epinephrin, and norepinephrin (Lindquist, 1973). Selegiline and other amphetamines, chloroquine, dopamine, norepinephrin, and the morphine derivative MPTP, can bind to melanin granules (Bathory et al, 1987; Stepien et al, 1987; Lindquist 1973; Lindquist et al, 1987). Debing et al (1988) reported the binding of 15 aromatic drugs to calf eye melanosomes and to synthetic dopa-melanin.

The binding of estrogens to melanin of melanoma could be responsible for the favorable effect associated with estrogens during the later course of development of the malignancy in women. Women with melanoma, especially during the child-bearing age, have a better survival rate than men. The effect may be due to oxidative reactions of estradiol leading to formation of ortho-quinones which, by acting as melanocytotoxic agents, could provide the host with a mechanism for defense. Precedence exists for the action of quinones as cytotoxic intermediaries. A specific strategy for treatment of melanoma is based upon tyrosinase activation of pro-drugs, with selectivity conferred by the enzyme (Wick 1983; Pezzuto et al, 1988). Quinones and semiquinones are generated and it is assumed that semiquinones act biologically via generation of superoxide (Pezzuto et al, 1988). The cytotoxicity of 4-hydroxyanisole to malignant melanoma was proposed to be due to its oxidation to the ortho-quinone and subsequent reverse dismutation to the semiquinone, but it is uncertain whether the quinone or the semiquinone is the actual cytotoxic product (Riley 1985; Nilges et al, 1984). In studies on malignant cells in culture, estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol were

found to be cytotoxic to dividing MCF-7 and HeLa cells (Seegers et al, 1989). On exposure of these cells to high concentrations ($\geq 1 \times 10^{-6}$ M) of the steroids, they showed increased mitosis with formation of abnormal and fragmented polar bodies and disoriented microtubule arrangements (Seegers et al, 1989).

Tyrosinase, the enzyme responsible for melanin formation by way of successive oxidations of tyrosine and its products, has the ability to oxidize estrogens (Jellinck et al, 1971; Jellinck et al, 1963; Jacobsohn et al, 1988; Jacobsohn et al, 1984). Mushroom tyrosinase has been used for some time in the laboratory preparation of 2-hydroxyestradiol from estradiol (Jellinck et al, 1971; Hersey et al, 1981), and we found that the enzyme can oxidize the hydroxylated product in stoichiometric relationship to molecular oxygen in presence of catechol (Jacobsohn et al, 1984). The enzyme used in this work originated from mushrooms but it is likely that the mammalian enzyme can oxidize estrogens as well. Incubations of 2,4,6,7-^{3}H-estradiol with tyrosinase isolated from B-16 melanoma cells yielded ^{3}H-water (Ewaskiewicz and Jacobsohn, in prepn.) by methodology analogous to the Pomerantz assay for tyrosinase activity (Pomerantz, 1969). Incubations of [^{14}C]-estradiol or [^{14}C]-2-hydroxyestradiol, together with DOPA and tyrosinase from the B16 mouse melanoma cell line, produced melanin with the estrogen label firmly attached to the pigment (Jacobsohn et al, 1988). The steroids were attached to melanin in such a way that they were resistant to extraction with organic solvents such as ethyl ether or hot methanol. This was taken as evidence that the unextractable portion of steroids had been oxidized and bound to melanin in covalent linkage; similar experiments with the mushroom enzyme have already shown that the 2-hydroxy estrogen can be oxidized and incorporated into melanin (Jacobsohn et al, 1988; Jacobsohn et al, 1984). An alternate but remote possibility is that the estrogens are bound to inner spaces within the pigmented polymer where the solvent cannot reach them. In more recent experiments, it was possible to isolate and identify the 2,3-orthoquinone of estradiol from incubation mixtures of the fungal enzyme and 2-hydroxyestradiol (Jacobsohn et al, 1989; Jacobsohn et al, 1990). Incubations of the quinone with murine tyrosinase and dopa have shown that the quinone can be incorporated into melanin, perhaps by additional enzyme-catalyzed steps or through reverse dismutation with dopa-melanin intermediates (Jacobsohn et al, 1990). It should be noted that melanin itself, in the absence of tyrosinase, may function as an oxidant for estrogens, because exposure of melanin to estradiol or 2-hydroxyestradiol can cause physiologically significant amounts of steroid to be retained by the pigment (Jacobsohn et al, 1988; Jacobsohn et al, 1990).

Estrogens are thought to be co-carcinogens because they are able to exacerbate the activity of true carcinogens but do not appear to initiate growth of a malignancy by themselves. After the growth of melanoma cells has been triggered by external events, estrogens may intensify the malignancy by binding to melanin of affected cells. This may explain the often-noted higher incidence of melanoma in women compared to men. As the disease progresses and increased amounts of estrogens are concentrated in melanoma cells, their conversion to cytotoxic oxidation products may present a more favourable outcome. We hope that this hypothesis will stimulate additional research on the relationship of estrogens to growth and development of pigment cells.

References

Bathory G, Szuts T, Magyar K: Pol J Pharmacol Pharm, 39, 195-201, 1987

Debing I, Ijzeman AP, Vauquelin G: Molec Pharmacol, 33, 470-476, 1988

Hersey RM, Williams KIH, Weisz J: Endocrinology, 109, 1912-1920, 1981

Jacobsohn GM, Jacobsohn MK: Arch Biochem Biophys, 232, 189-196, 1984

Jacobsohn GM, Chiartas PL, Hearing VJ, Jacobsohn MK: Biochim Biophys Acta, 966, 222-230, 1988

Jacobsohn GM, Jacobsohn MK, Enu B, Byler DM: The Endocrine Society, 71st Annual Meeting, 1403, 1989

Jacobsohn MK, Dobre VC, Branam C, Jacobsohn GM: J Steroid Biochem, 31, 377-385, 1988

Jacobsohn MK, Byler DM, Jacobsohn GM: submitted

Jellinck PH, Irwin L: Biochim Biophys Acta, 78, 778-780, 1963

Jellinck PH, Brown BJ: Steroids, 17, 133-140, 1971

Lindquist NG: Acta Radiol, Suppl. 325, 325, 67-82, 1973

Lindquist NG, Larsson BS, Lyden-Sokolowski A: Pigment Cell Res, 1, 133-136. 1987

Lyttkens L, Larsson BS, Stahle J, Englesson F: Adv Oto-rhino-laryng, 25, 17-25, 1979

Lyttkens L: Scand Audiol, Suppl., 26, 27-31, 1986

Nilges MJ, Swartz HM, Riley PA: J Biol Chem, 259, 2446-2451, 1984

Pezzuto JM, Shieh HL, Shaughnessyy E, Beattie CW: Sem Oncol, 150, 578-588, 1988

Pomerantz SH: Science, 164, 838-839, 1969

Riley PA: Phil Trans R Soc Lond B, 311, 679-689, 1985

Seegers JC, Aveling ML, Van Aswegen CH, Cross M, Koch F, Joubert WS: J Steroid Biochem, 32, 797-809, 1989

Stepien K, Porebska M, Wilczok T: Studia Biophys, 122, 165-174, 1987

Wick MM: J Invest Dermatol, 80 (suppl.), 61s-62s, 1983.

****

D I S C U S S I O N

Analysis of cancer early processes initiation in Xiphophorus melanoma model (Ansätze zur Analyse der Initiation von Initialprozessen der Krebsbildung am Melanom-Modell von Xiphophorus)

A. Anders, F. Anders, Ch. Zechel, U. Schleenbecker, A. Smith
Genetisches Institut der Justus-Liebig-Universität Giessen, 6300 Giessen, FRG and Dept of Chemical Pathology, University College and Middlesex School of Medicine, London W1P 6DB, UK

Certain backcross hybrids (BC_{8-22}) of a spotted X.maculatus

(platyfish) and a non-spotted X. helleri (swordtail; recurrent parent) are highly sensitive to mutagenic carcinogens and, after a latent period of 8 to 12 months, develop melanoma of unicellular origin that is genealogically related to the spots of the platyfish. Sensitivity to the carcinogen or susceptibility to melanoma, respectively, are inherited in a Mendelian fashion and can be assigned to a "tumor gene-complex" (Tu-complex) consisting probably of almost 20 genes. The Tu-complex is located at the end of an autosome or sex chromosome, and is largely deregulated by crossing conditioned replacement of platyfish chromosome carrying regulatory genes (tumor suppressor genes, oncostatic genes, antioncogenes) for the Tu-complex by swordtail chromosomes lacking them. The melanoma-free condition of these BC-hybrids depends upon the skin-specific regulatory gene Bs (body side) that requires impairment in a pigment cell precursor for the outgrowth of melanoma.

Structural mutations involving different breakpoints indicate that the signal for melanoma formation comes from a particular region of the Tu-complex where an accessory v-erbB related oncogene (v-erbB*^{a}; 85% homology to the human EGF receptor gene) is located. Northern blot analyses of a melanoma cell line showed an about 20-fold overexpression of x-erbB*^{a}. Both the inositol lipid turnover ((^{3}H)inositol incorporated into phosphoinositides), and the xiphophorine pp60$^{x\text{-}src}$ kinase activity that are assumed to be causally involved in tumor formation showed a remarkable elevation in the melanoma as compared to the normal tissue (brain) of the tumorous and non-tumorous (with or without the Tu-complex) segregants.

Other BC hybrids carrying the Tu-complex but lacking the linked regulatory gene develop melanoma "spontaneously". This kind of melanoma occurres early in the course of life, is of multicellular origin, and is inherited as a Mendelian character. In contrast to the BC hybrids requiring somatic mutation for melanoma formation, both inositol lipid turnover and x-src activity are remarkably enhanced in both melanoma and normal tissues.

A mutant of the latter BC hybrids carrying in addition to the Tu-complex the homozygous oncostatic gene g (g/g, "golden") that arrests pigment cell differentiation in the stem cell stage is incapable to develop melanoma spontaneously. Nevertheless it shows the elevation of inositol lipid turnover and x-src activity in its always healthy tissues. Following treatment with tumor promoters such as TPA and steroid hormones pigment cell differentiation recovers and melanoma of multicellular origin develops within 4 to 8 weeks. This kind of melanoma adopts the elevated inositol lipid turnover and x-src activity of the normal tissue.

Another mutant of the BC hybrids carrying a Tu-complex that has lost a distal gene coding for pigment cell differentiation but has retained the x-erbB*^{a} showed, although incapable of melanoma development, the same elevation of inositol lipid turnover and x-src activity in the brain.

In contrast, a phenotypic similar mutant of the BC hybrids that, in addition to the loss of information for pigment cell differentiation (and for melanoma development) has lost the erbB*^{a} gene, shows a resting phosphoinositide inositol turnover and a resting pp60$^{x\text{-}src}$ kinase activity in the normal tissues.

Our results indicate an enhancement of phosphatidyl

inositol lipid turnover and an elevation of pp60x-src kinase activity in all melanomas tested. This elevation is intimately linked with the inheritance and probably with the expressionof x-erbB*a. If this gene is present in the genome of the hybrids carrying the deregulated Tu-complex the proposed molecular and biochemical machinery of melanoma formation may be running by genetic reasons with or without forming melanoma; whether melanoma develops or fails to develop, respectively, depends upon the presence or lack of cells competent for transformation. If, however, the machinery is resting because of the lack or control of x-erbB*a containing region of the Tu-complex, no melanoma develops irrespective of whether the competent cells are present or lacking. It appears that x-erbB*a, x-src, and inositol lipid turnover might be involved in key processes preceding melanoma formation in Xiphophorus.

****

LETTER TO THE EDITOR OF

PIGMENT CELL BULLETIN

Dr Manfred Schartl, Gene Center, Max-Planck-Institute for Biochemistry, Martinsried, Fed. Rep. Germany

In the May issue (Nr. 9) of the Pigment Cell Research Bulletin a commentary by Zechel C, Schleenbecker U, Anders A, and Anders F and by the editor was published stating that the work of our group (Wittbrodt et al, Nature 341:415-421, 1989), dealt with the same subject, that we also found an EGF (receptor) related Tu-gene, but that we did not quote the previously published work by Zechel et al (Oncogene 3:605-617, 1988). This implicates : 1) our manuscript and that of Zechel et al contain the comparable experimental work and results; 2) the work of Zechel et al is the first report of its kind; 3) our group does not quote correctly important work in the same area; 4) the group of Prof. Dr. Anders, Giessen, has first reported that the long sought Tu-gene of Xiphophorus is an EGF-receptor like gene.

My collaborators and I disagree to that. Our reasons for that are:

1 The manuscript of the Anders group describes a v-erb B related sequence, which cosegregates with certain sex-chromosomes that harbour a Tu-locus, cloning of this sequence, a partial nucleotide sequence (approx. 500 bp in total, coding 250 bp) and its expression in two established cell lines.

Our manuscript demonstrates that such a sequence is not only closely physically linked to Tu, but the critical constituent of this locus. We describe cloning and genomic organization of more than 45 kb of the corresponding loci, show its simultaneous presence as proto-oncogene and oncogene in the genome of susceptible fish, isolation and sequence of a full length c-DNA (approx. 4000 bp thereby proving that it is a function al gene), show that this gene is a novel putative receptor tyrosine kinase, its differential expression during embryonic development, and its strongly enhanced and aberrant expression in 12 different samples from melanoma biopsies. Central and most important for our paper is the analysis of a loss of function mutant, which we showed to be due to the insertional inactivaiton of the gene that we have described, being so far the single experimental proof that this novel receptor tyrosine kinase is indeed the melanoma inducing gene encoded by the Tu-locus.

We are confident that the manuscript of Zechel et al and our manuscript do not contain comparable work.

2 The cloning, the linkage and

extended expression data not only in cell lines but also in a variety of normal tissues and in melanoma of a v-erb B related sequence was published by our group in a total of four manuscripts all of which appeared prior to the paper by Zechel etal (Dec. 88). These are : Mäueler et al, Oncogene 2:421-430, 1988; Schartl, Genetics 119:679-695, 1988; Adam et al, Nucl Acid Res 16:7212, 1989, Mäueler et al, Oncogene 3:113-122, 1988. None of these have been quoted in the paper by Zechel et al.

3 In our article when we were referring to some earlier work that has led us to the isolation of Tu, we made use of our own original data rather than using the confirmatory work of others. If there is something in our article which may look related to the work of Zechel et al to somebody from other fields in research, this may be contained on page 415 in the last paragraph. We would, however, like to stress that our experiment described there is a linkage analysis of the cloned marker sequence and one specific Tu-allele (Tu-Sd) and we cannot find such experiment in the paper of Zechel et al.

4 Concerning the claim that the Tu-gene is a EGF-receptor (or v-erb B) related gene we would like to add that in the paper by Zechel et al the authors state explicitely a) that the v-erb B related sequences "are located between the respective pterinophore locus ... and the Tu complexes ..." b) that the v-erb B related sequences "do not mediate the process of neoplastic transformation itself, but are rather involved in other processes such as determination of the target cells ..." c) "The biological nature of the formally defined tumor gene Tu still remains unknown".

On the contrary, our group has always suggested (e.g. see our papers from 1988) and finally conclusively proven that the v-erb B related sequence which we have designated Xmrk is the Tu gene (see Wittbrodt et al).

****

CURRENT LITERATURE IN PIGMENT CELL RESEARCH

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

1. MELANINS AND OTHER PIGMENTS CHEMISTRY

- Bigelis R, Black KA.
Manufacture of homogentisic acid and melanin from tyrosine and phenylalanine using Yarrowia lipolytica. U.S., 4 pp., 1989.
Abstract : Growth of Yarrowia lipolvtica YV3-180 on a poor C-source in the presence of phenylalanine or tyrosine results in the formation of homogentisic acid. Melanin is formed, if desired, by bringing the medium to pH >10 whereupon the homogentisic acid spontaneously polymerizes. Y. lipolytica YB3-180 was grown in a minimal salts medium contg. 0.5 g glucose/L and tyrosine 5 mM. After 5 days incubation (28.degree., with shaking) 40% of the tyrosine had been converted to homogentisic acid that was recovered by solvent extn. of the acidified medium. Alternatively, the medium was brought to pH 10 with NH4OH with the rapid formation of melanin. Wild-type strains produced neither compd. efficiently.

- Bogacz A, Buszman E, Wilczok T.
Competition between metal ions for DOPA-melanin. Stud Biophys 132:189-195, 1989.
Abstract : Metal ions (Zn2+, Mn2+, Co2+, Fe3+, and Cr3+) were shown by radiochem. anal. to be bound to synthetic DOPA-melanin in different amts. when administered as single cations or in mixts. of 2 metal ions. The following affinity was found: Fe3+ > Cr3+ > Co2+ > Zn2+ > Mn2+ when binary metal ion-melanin complexes were examd. In ternary systems (2 metal ions/melanin) the highest binding capacity was detected for Fe3+. The competition between metal ions for melanin was described for 10 pairs of metal ion-melanin ternary complexes.

- Bubnova E, Budesinska A, Schwippelova Z, Matous B, Trnka T.
Synthesis of eumelanin pigment precursors. II. 6-Hydroxy-5-methoxy- and 5-hydroxy-6-methoxyindol. Sb Lek 91:225-229, 1989.
Abstract : Following up of specific melanogenesis metabolite excretion in the course of malignant melanoma disease is useful for the disease prognosis assessment. The authors elaborated a modified synthesis technique of 6-hydroxy-5-methoxy and 5-hydroxy-6-methoxyindole, eumelanin pigment precursors. The elaborated synthesis is economically and temporally reasonable and the synthesized compounds accord, as proved by means of elementary analysis, thin layer (TLC) and high performance liquid chromatography (HPLC) and last but not least by nuclear magnetic resonance (NMR), with demands for

reference compounds needed for isomer hydroxymethoxyindole quantification in urine.

- Clark MB Jr, Gardella JA Jr, Schultz TM, Patil DG, Salvati L Jr.
Solid-state analysis of eumelanin biopolymers by electron spectroscopy for chemical analysis. Anal Chem 62:949-956, 1990.
Abstract : Electron spectroscopy for chem. anal. (ESCA or XPS)) was used to elucidate the chem. compn. and org. functional group distribution within 1 type of biopolymer, eumelanin. Qual. and quant. elemental analyses were calibrated with model compds. In addn., the relative proportions of the different C, O, and N functionalities within these eumelanin precursor model compds. and the polymers generated from them are presented. The ESCA results showed that eumelanin has structural and compositional components consisting of both arom. and partially oxidized units of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid.

- Crippa PR, Viappiani C.
Photoacoustic studies of nonradiative relaxation of excited states in melanin. Eur Biophys J 17:299-305, 1990.

- Crippa R, Horak V, Prota G, Svoronos P, Wolfram L.
Chemistry of melanins. Alkaloids (Academic Press) 36:253-323, 1989.

- Galvao DS, Caldas MJ.
Theoretical investigation of model polymers for eumelanins. I. Finite and infinite polymers. J Chem Phys 92:2630-2636, 1990.
Abstract : The electronic structure was studied of ideal ordered oligomers of 5,6-indolequinonein one of its redox forms. The study is carried out through Hueckel .pi.-electron theory, which allows one to follow the trends in electronic structure from a single monomer, the isolated mols., to finite polymers of .ltoreq.10 units, and to infinite polymers. Different polymn. directions were chosen which produce semiconducting chains. The comparison between finite and infinite polymers is very useful and allows proposal of a model that accounts for some of the known properties of eumelanins.

- Kajiwara M, Kurumaya K, Kohno Y, Tomita K, Carpenter AT.
Stereochemical studies on the formation of melanin by monophenol monooxygenase. Chem Pharm Bull (Tokyo) 37:3386-3389, 1989.

- Karpinski ZJ.
The effect of copper(II) ions on dopaquinone cyclization. Bioelectrochem Bioenerg 21:261-268, 1989.
Abstract : The kinetics of the dopaquinone (I) cyclization in the absence and presence of Cu(II) ions at pHs from 6 to 7.4 has been studied by cyclic, normal and reverse pulse voltametry. Distinct inhibition of the dopaquinone ring closure reaction was obsd. in the presence of Cu(II) ions. At pHs below 6, this effect is attributed to the formation of amino acid type complexes. At pH 7.4 the amino acid type and the catechol type CunII)-DOPA chelates coexist, and simultaneous interactions of copper ions with both ends of the DOPA mol. result in the assocn. of the Cu(II)-DOPA complexs. These effects, obsd. at physiol. pH, suggest that the rate of melanin formation is affected by the presence of Cu(II) ions.

- Kiel JL, O'Brien GJ, Dillon J, Wright JR.
Diazoluminomelanin: a synthetic luminescent biopolymer. Free Radic Res Commun 8:115-121, 1990.
Abstract : The purpose of this work was to synthesize a water-soluble derivative of 5-amino-2,

3-dihydro-1, 4-phthalazinedione (luminol) that generated sustained high level luminescence under physiologic conditions without the necessity of a catalyst. The derivative was made by a diazotization reaction with luminol and 3-amino-L-tyrosine. The resulting orange-brown anionic polymer has been given the trivial name of diazoluminomelanin (DALM). It was water soluble above and insoluble at or below pH 5.0. DALM luminesced when treated with hydrogen peroxide without the presence of a catalyst at pHs ranging from 6.5 to 12.0. Microgram quantities produced high levels of chemiluminescence for longer than 52 hr. Dried polymer generated a long-term stable electron spin resonance spectrum. The long-term chemiluminescence of DALM at pH 6.8-7.4 makes it a potentially useful reagent for detecting free radicals and peroxides in cellular and biochemical preparations.

- Motono M.
Manufacture of topical cosmetics and pharmaceuticals containing saponins as absorption accelerators. Jpn. Kokai Tokkyo Koho, 5 pp., 1989.
Abstract : A topical cosmetic or pharmaceutical contains saponin as drug absorption accelerator and .gtoreq.1 physiol. active agent such as melanin-formation inhibitors (kojic acid, vitamin C, hydroquinone, a placenta ext., etc.), indomethacin, glycyrrhetinic acid, flurbiprofen, ibuprofen, scopolamine, nitroglycerin, estradiol, hinokitiol, minoxidil, and vitamins. Thus, a skin lotion was prepd. contg. 1% by wt. glycyrrhetinic acid and 0.6% saponins.

- Motono M.
Manufacture of topical cosmetics and pharmaceutical containing ginger extracts as absorption accelerators. Jpn. Kokai Tokkyo Koho, 5 pp., 1989.
Abstract : A topical cosmetic or pharmaceutical contains a ginger ext. as drug absorption accelerator and .gtoreq.1 physiol. active agent such as melanin-formation inhibitors (kojic acid, vitamin C, hydroquinone, a placenta ext., etc.), indomethacin, glycyrrhetinic acid, flurbiprofen, ibuprofen, scopolamine, nitroglycerin, estradiol, hinokitiol, minoxidil, and vitamins. Thus, a skin lotion was prepd. contg. 1% by wt. glycyrrhetinic acid and 0.1% of a ginger ext.

- Novellino E, Mayol L, Chioccara F, Bavoso A, Santini A, Pedone C.
Crystal structures of two trimers of 1,4-benzothiazine. Heterocycles 29:1669-1673, 1989.
Abstract : Crystal and mol. structures of two diastereoisomeric [1R,2S,3R,4S,5S and 1S,2R,3S,4R,5R] trimers of 1,4-benzothiazine I, the parent system of the key intermediate in biosynthesis of phaeomelanins, were unambiguously detd. by NOE expts. and x-ray anal. The relative configuration of the asym. centers has been detd. The two compds. show inverted configurations at the chiral atom C(4) leading to different overall shapes of the mols.

- Osak W, Tkacz K, Czternastek H, Slawinski J.
I-V characteristics and electrical conductivity of synthetic melanin. Biopolymers 28:1885--1890, 1989.
Abstract : I-V characteristics for synthetic melanin were investigated at -10 and 25.degree.. The Ohm law is valid for low voltages whereas the Child law holds for higher ones. Furthermore, the steady state elec. cond. was estd. The change of activation energy from 0.76 eV for low temps. to 1.58 eV was noticeable near 0.degree..

- Oyama Y.

Mercaptopyridine for melanin-formation prevention. Jpn. Kokai Tokkyo Koho, 5 pp., 1989.
Abstract : Mercaptopyridine is useful for prevention of melanin formation. 2-Mercaptopyridine (I) at 20 .mu.M exhibited a remarkable skin lightening effect. A cosmetic lotion was formulated contg. poly(oxyethylene) castor oil 1.00, EtOH 15.00, citric acid 0.10, Na citrate 0.30, 1,3-butylene glycol 4.00, I 0.50, antiseptic agent, perfume, and H2O to 100% by wt.

- Oyama Y
Gray hair-preventing agents containing unsaturated fatty acids or their esters. Jpn. Kokai Tokkyo Koho, 3 pp., 1989.
Abstract : Gray hair-preventing agents contain unsatd. fatty acids CH2=CH(CH2)nCO2R (R = H, C1-2 alkyl; n = 7-10) as active ingredients. The fatty acids promote the formation of melanin. A treatment oil was prepd. from jojoba oil 20.0, mink oil 20.0, 10-undecylenic acid 1.0, fragrance, and squalane to 100 g.

- Parvez M, Kurtz SK, Williams I.
Structure of a melanin precursor: 1-methylindole-5,6-diol. Acta Crystallogr, Sect. C: Cryst Struct Commun C46:165-166, 1990.
Abstract : The title compd. is rhombohedral, space group R3c, with a 12.814(4) .ANG. and .alpha. 114.59(2).degree.; dc = 1.332 for Z = 6. The final R = 0.027 for 410 reflections. At. coordinates are given. The C-O distances in the catechol are identical. The indole moiety is planar with O(1) 0.139 .ANG. out of the plane of the indole moiety. The structure is stabilized by 2 short intermol. O...H distances and there is a short intramol. O...H contact.

- Raghavan PR, Zane PA, Tripp SL.
Calculation of drug-melanin binding energy using molecular modeling. Experientia 46:77-80, 1990.
Abstract : Conformational analysis and molecular graphics are used to model a representative melanin structure to estimate a chemical's in vitro affinity for melanin. The modelling data fit to a simple linear equation relative to a logarithmic transformation of the experimentally-derived binding data (r = 0.901). The goodness of fit, as evidenced by the F-statistic, F (1,14) = 60.09 (p = 0.000002), for the regression indicates that this technique gives an accurate representation of the interaction of these chemicals with melanin in vitro.

- Salazar-Bookaman MM, Fowble J, Weber P, Patil PN.
Investigation by NMR spectroscopy of the interaction between synthetic soluble (-)-dopa melanin and drugs. Naunyn Schmiedebergs Arch Pharmacol 340:576-582, 1989.
Abstract : In order to understand the molecular interactions of drugs with melanin, synthetic soluble (-)-dopa-melanin was prepared in deuterium buffer. The spectra of various drug moieties with the pigment at 30 degrees C were studied employing the line width measurements obtained with a pulse NMR (AF270) instrument. As compared to drug effects in fresh melanins (48 h), the aged melanins (greater than or equal to 168 h) gave consistent spectral measurements, even in dilute solutions of pigment. NMR signals of aromatic and N-methyl protons of drugs were relatively easy to quantify and, in the presence of melanin, line broadening of various drug moieties occurred. The line widths of the N-methyl groups of acetylcholine (3.02 ppm), the N-methyl group of atropine (2.52 ppm), N-isopropyl of isoprenaline bitartrate (1.14 ppm) and N-ter-butyl of timolol maleate (1.22 ppm) in the presence of the

pigment were increased. Line widths associated with acetate, bitartrate, maleate or tropic acid, however, were not altered by the melanin. This indicates the specificity of the interaction between drug moieties and the site(s) of melanin. Based on the line width measurements of N-methyl protons of ephedrine, two dissociation constants were obtained (Kd1 2.08 mM and Kd2 greater than 20 mM). The constants for atropine melanin complex were Kd1 0.79 mM and Kd2 greater than 6 mM. Furthermore, based on N-methyl resonances, it appears that atropine and ephedrine compete for at least one common interacting site of the melanin polymer.

- Singh S, Jen JF, Dryhurst G. Autoxidation of the indolic neurotoxin 5,6-dihydroxytryptamine. J Org Chem 55:1484-1489, 1990.
Abstract : The autoxidn. of 5,6-dihydroxytryptamine has been studied in pH 7.2 phosphate buffer. The major initial product of the autoxidn. has been isolated and, by using spectral methods, its structure is shown to be 2,7'-bi(5,6-dihydroxytryptamine), a likely precursor to melanins.

2. BIOLOGY OF PIGMENT CELLS AND PIGMENTARY DISORDERS

- Cegarra J, Gacen J, Schumacher-Hamedat U, Knott J, Blankenburg G, Caro M.
Depigmentation of animal hair. Rev Quim Text 96:26-31, 333638-40, 1989.
Abstract : Alpaca, yak, and camel hair samples were treated using various methods to remove pigments and produce light colored hair for textile manuf.; FeSO4-based mordant baths and bleaching under reducing conditions were evaluated. The selective destruction of pigments was catalyzed by Fe2+ which formed complexes with melanin, upon addn. of bleaching agent (H2O2, Na2S2O4, H3PO3, etc.). The process required long periods of rinsing with water to remove free Fe2+ and addn. of complexing agents such as EDTA and NTA.

- Czapla TH, Hopkins TL, Kramer KJ.
Catecholamines in the cuticles of four strains of the German cockroach Blattella germanica (L.) during sclerotization and melanization. Arch Insect Biochem Physiol 12:145-156, 1989.
Abstract : Catecholamines were extd. from the cuticles of 4 strains of the cockroach B. germanica at different times 48 h after adult ecdysis and analyzed by reverse-phase HPLC with electrochem. detection. The wild (VP1), black (B1), orange (or), and yellow (y) phenotypes differ in cuticular pigmentation, particularly in the extent of melanization. N-.beta.-Alanyldopamine (NBAD) and N-.beta.-alanylnorepinephrine (NBANE) were major o-diphenolic compds. in exts. from cuticle of all strains during the main period of sclerotization. N-Acetyldopamine (NADA) and N-acetylnorepinephrine (NANE) were minor the 1st day after ecdysis, but accumulated to higher levels thereafter. Dopamine (DA) concns. were higher in the darker pigmented cuticles of strains B1 and or than in the lighter-colored cuticles of strains VP1 and y. Extractable DA rapidly increased in VP1, B1, and or cuticles shortly after ecdysis, reached peak levels 6-24 h later, and then decreased after

melanization. Only small amts. of DA were detected in strain y cuticle, whereas NBANE concns. were very high. Therefore, high DA levels in cuticle are correlated with melanization that occurs during the 1st few hours after adult ecdysis, whereas sclerotization is correlated with high levels of the N-.beta.-alanylcatecholamines. Sclerotization appears to be delayed in strain Bl, since only low concns. of the N-acylated catecholamines accumulate until after melanization is completed.

- Greenberg RG, Berger TG.
Nail and mucocutaneous hyperpigmentation with azidothymidine therapy. J Am Acad Dermatol 22:327-330, 1990.
Abstract : Hyperpigmentation developed in six patients while they were receiving azidothymidine. All demonstrated hyperpigmentation of the nails; hyperpigmentation of the skin (two patients) and oral mucosa (two patients) also developed. The degree of nail pigmentation was related to the intrinsic skin color of the patient. Mucosal hyperpigmentation developed only in dark-skinned blacks. The pigmentation was due to increased melanin in the epidermis and dermis.

- Guerra L, Bover L, Mordoh J.
Differentiating effect of L-tyrosine on the human melanoma cell line IIB-MEL-J. Exp Cell Res 188:61-65, 1990.
Abstract : IIB-MEL-J is a highly heterogeneous newly established human melanoma cell line. The addition of 3 mM L-tyrosine to the culture medium produced (1) a great decrease in the cell growth rate, (2) a loss of the anchorage-independent growth capacity, and (3) a change in the morphology of the cells to a fibroblastoid aspect. Coincident with these changes, an increase in subpopulations I and II and a decrease in subpopulations III and IV took place. In view of this evidence we consider that the cells have differentiated. The melanin production was not increased by the L-tyrosine treatment, suggesting that differentiation and melanin expression are not strictly correlated.

- Hayashiba Y, Ikeda H, Iki H, Kita T, Furuya Y.
Transmission electron microscopy of the consumed hairs in an Australian lobster's stomach. Igaku Kenkyu 59:15-18, 1989.
Abstract : The present report is concerned with the transmission electron microscopic observations of the consumed hairs in an Australian lobster's stomach. The lamellar cuticles of these hairs were observed and their minimum diameters of melanin granules of cortices were measured by an imaging analyzer. The lamellar cuticles of these hairs consisted of two or three layers and there was no significant difference between them and Aberdeen Angus's hairs in mean the minimum diameters of melanin granules of cortices. Consequently, these hairs in an Australian lobster's stomach were identical to Aberdeen Angus's hairs.

- Herzberg AJ, Kaplan DL.
Cronkhite-Canada syndrome. Light and electron microscopy of the cutaneous pigmentary abnormalities. Int J Dermatol 29:121-125, 1990.
Abstract : This article describes the light and electron microscopic studies from a macule and the surrounding lightly hyperpigmented skin of a patient with the Cronkhite-Canada syndrome. Increased numbers of melanin granules in keratinocytes, increased numbers of melanosomes in melanocytes, and areas with increased numbers of melanocytes were found in the macular lesion. Skin from both the macule and

surrounding area were also characterized by compact hyperkeratosis as well as perivascular inflammation and exocytosis not previously reported in these patients.

- Kreusch J, Rassner G.
Structural analysis of melanocytic pigment nevi using epiluminescence microscopy. Review and personal experiences. Hautarzt 41:27-33, 1990.
Abstract : Epiluminescent microscopy is now used frequently for the differential diagnosis of pigmented skin lesions. In order to improve the distinction between benign and malignant melanocytic tumours it seemed advisable to develop a standardized pathway of epiluminescent microscopical analysis and to determine the frequency of structures recognizable with the microscope. A total of 600 melanocytic lesions were examined by epiluminescent microscopy, photographed and classified histologically after excision, revealing 426 naevocellular naevi and 174 melanoma. The experience achieved during the course of the investigation was used as the basis of a procedure for stepwise analysis of keratin layer, pigment structures and blood vessels. The most important findings are described and referred to malignancy. The photographs were analysed for the frequency of occurrence of various pigment structures in different types of lesions. The results differ in several aspects from previous findings.

- Meybeck A, Dumas M.
Hydrated lipidic lamellar composition or liposome containing tyrosine or tyrosine derivative for tanning composition. Eur Pat Appl, 10 pp., 1989.
Abstract : Hydrated lipid lamellar phases or liposomes, that incorporate tyrosine, or its salts or esters, are prepd. as suntanning agents and hair-graying-inhibitors. L-Tyrosine (0.2g) was dispersed into a soln. of 1.8g hydrogenated soybean lecithin and 0.06 g .alpha.-tocopherol in 20 mL Cl2CH2. Following solvent evapn., the residue was suspended in 98g phosphate buffer (pH 7.5), following by gelling with Cabopol 940, to give a liposome compn. The melanin formation-stimulating effect of this gel was demonstrated on the rat skin, in vivo. A suntanning compn. comprised the gel 100, soybean lecithin 3.8, .beta.-sitosterol 0.2, .alpha.-tocopherol 0.1 and com. glucose tyrosinate 1g.

- Mukai H, Nishioka K, Kamimura K, Katayama I, Nishiyama S.
Poikiloderma-like lesions on the neck in atopic dermatitis: a histopathological study. J Dermatol 17:85-91, 1990.

- Nakano M.
Clinical application of cultured autologous epithelium to donor sites for split-thickness skin graft. Hokkaido Igaku Zasshi 65:56-66, 1990.
Abstract : The present study investigated the interaction between the in situ dermis by using the donor site for split-thickness skin graft (STSG) and the cultured autologous epithelium in eleven individuals. Human epithelial cells were cultured according to the method of Rheinwald and Green, with some modification. The mirror-image site was covered by ointment dressing, and used as control. All of the grafted epithelial adhered to the wound bed within 7-8 days, and they were thick enough to be manipulated. On the other hand, the control areas didn't re-epithelize until 13-18th day postoperatively. Pain and exudate were remarkably reduced in all the cases, and there was less itching in 9 cases, as compared to the control

areas (P less than 0.01; sign test). Even though there was no visual difference of scarring after 3 months, the grafted areas were much softer than the control areas in 8 cases (P less than 0.01). There was also a definite difference concerning pigmentation decrease in 5 cases. 7 days post-grafting, the cultured epithelium became thicker and more differentiated in 8-10 cell layers, and a well-developed basal lamina was observed by PAS stain and on electron micrographs. A 14th day specimen from the grafted area showed almost normal epidermis with melanin granules in the basal layer, despite of a mild intercellular edema among basal and spinous cells. Biopsies after 3 months revealed that there was less dermal fibrosis in the grafted areas than in the ungrafted ones. There appeared a tendency of rapid healing associated with minimal fibrosis, leading to satisfactory results. It is possible to infer that the epithelial-dermal interaction induced by cultured epithelial autograft may influence and regulate the formation of collagen and other extracellular matrix by fibroblasts. This study suggests that cultured autologous epithelium can provide a successful permanent skin substitutes in the donor site for STSG.

- Novales RR, Davis WJ.
Melanin-dispersing effect of adenosine 3',5'-monophosphate on amphibian melanophores. Endocrinology 81:283-290, 1967.

- Orlow SJ, Chakraborty AK, Pawelek JM.
Retinoic acid is a potent inhibitor of inducible pigmentation in murine and hamster melanoma cell lines. J Invest Dermatol 94:461--464, 1990.
Abstract : Melanocyte-stimulating hormone (MSH) induces melanogenesis in Cloudman mouse melanoma cells. The activities of two enzymes in the melanogenesis pathway, tyrosinase and dopachrome conversion factor, are increased as part of the induction process. Trans retinoic acid (RA), at concentrations as low as 0.1 nM, inhibited the induction of tyrosinase, dopachrome conversion factor, and melanogenesis, but had no effect on the basal levels of either enzyme or of cellular melanin content. Half-maximal effects of RA occurred at a concentration of 10 nM; maximal effects were observed at 1 microM. The effects of RA on melanogenesis were independent of its effects on cellular growth since one Cloudman line tested was growth-inhibited by RA and another was growth-stimulated by RA, but the induction of melanogenesis by MSH in both lines was inhibited by RA. Mixing experiments with cell lysates failed to demonstrate the induction of a tyrosinase inhibitor by RA. The effects of RA were not limited to MSH or to Cloudman melanoma cells since RA blocked cholera toxin-inducible melanogenesis in Cloudman cells, as well as the induction of tyrosinase activity by L-tyrosine in Bomirski hamster melanoma cells. The effects of RA were specific to melanogenesis, however, since RA did not interfere with MSH-induced changes in cellular morphology and growth. Thus, RA appears to be a new and potent tool for understanding mechanisms regulating induction of the pigmentary system.

Ortonne JP.
Melanogenesis 1989: biochemical and cellular aspects. Colloq INSERM 201:143-157, 1989.
Abstract : A review, with 56 refs., on melanin biosynthesis, melanocyte-keratinocyte interactions, and the regulation of melanogenesis by growth factors, hormones, vitamins, mediators of

inflammation, UV radiation, culturing of pathol. melanocytes, and monoclonal antibodies.

- Oyama Y.
Thiazolium derivative as melanin formation activator for prevention of gray hair. Jpn. Kokai Tokkyo Koho, 3 pp., 1989.
Abstract : A thiazolium deriv. (I) is useful for prevention of gray hair. I gives no damage to the skin and hair. I at 1.0 .mu.M activated melanoma B16 cells, resulted in melanin formation (38.86 .mu.g/106 cell, vs. 1.59 .mu.g/106 cell, for the control). A hair treatment oil comprised jojoba oil 20.0, mink oil 20.0, I 1.0, perfume, and squalane to 100

- Oyama Y, Oda K.
Nonadecenoic acids or eicosenoic acids as melanin formation activators for prevention of gray hair. Jpn. Kokai Tokkyo Koho, 3 pp., 1989.
Abstract : 7-Nonadecenoic acid (I), 8-eicosenoic acid, or their lower alkyl esters are useful for prevention of gray hair. Those compds. give no damage to the skin. I at 60 .mu.M activated melanoma B16 cell, resulted in 201% melanin formation, vs. 100%, for the control. A hair treatment oil comprised jojoba oil 20.0, mink oil 20.0, I 1.0, perfume, and squalane to 100 g.

- Pawelek JM.
Is human melanogenesis stimulated by cyclic AMP? J Invest Dermatol 94:499-500, 1990.

- Pillai S, Bikle DD, Elias PM.
Vitamin D and epidermal differentiation: evidence for a role of endogenously produced vitamin D metabolites in keratinocyte differentiation. Skin Pharmacol 1:149-160, 1988.
Abstract : Vitamin D3 is produced in the skin. It is metabolized primarily in the liver to the major circulating form 25-hydroxy vitamin D3, [25(OH)D3] which is metabolized in the kidney to produce the biologically active form of the hormone, 1,25-dihydroxy vitamin D3, [1,25(OH)2D3]. The skin not only participates in the production of vitamin D3 but also contains receptors for 1,25(OH)2D3 suggesting a role of this hormone in the growth and differentiation of this tissue. 1,25(OH)2D3 appears to play a role in epidermopoiesis and melanin pigmentation. Recently, using cultures of neonatal human foreskin keratinocytes, we have demonstrated that these cells produce abundant quantities of 1,25(OH)2D3 from 25(OH)D3. The production of 1,25(OH)2D3 varies with the degree of differentiation of keratinocytes and is regulated by exogenous 1,25(OH)-2D3. 1,25(OH)2D3 induces differentiation possibly because of its ability to increase intracellular free calcium levels. A tentative model is provided to demonstrate potential mechanisms by which 1,25(OH)2D3-induced changes in intracellular calcium could regulate epidermal differentiation.

- Roy P, Nayak KK, Pandey NK.
Characterization of a novel yeast synthesizing melanin-like pigment. J Gen Microbiol 135:-3385-3391, 1989.
Abstract : A lab. yeast isolated identified as Exophiala jeanselmei synthesized melanin and showed polyphenol oxidase activity with dihydroxyphenylalanine (DOPA) and pyrogallol. Electrophoresis on native and denaturing gels, and activity staining with DOPA revealed 2 immunol. distinguishable bands of Mr .apprx.100,000 and 120,000, which could be sepd. by (NH4)2SO4 fractionation. The enzyme isolated from this yeast gave optimal activity at higher pH (8.5-10) and temp. (37-40.de-

gree.) than other known melanin-synthesizing activities.

- Sugiyama K, Takada K, Yamamoto I.
Hair preparation containing cAMP derivatives. Eur Pat Appl, 17 pp., 1989.
Abstract : A compn. for preventing graying of the hair and restoring gray hair to its natural color comprises one member selected from the group consisting of adenosine 3',5'-cyclophosphoric acids (I or II; X = H, halo, S- or O-contg. group C1-12 alkylamino; Y = N, N-C1-12 alkyl; M = H, cation, provided that in I, X and Y do not simultaneously represent H and N, resp.). The compn. activates melanocytes of the radix pili and promotes synthesis of melanin. A hair tonic contg. I (X = SPh, Y = N, M = H) (III) 0.1, EtOH 85.5, polyethylene glycol-200 2, and water up to 100% was applied to stress-loaded mice (to cause gray hair); less gray hair was generated with the tonic than with the comparative compn. contg. N6,02--dibuytyl cAMP. After storage at 30.degree. for 3 mo, 100% III in the compn. was detected and no bad smell was sensed.

- Trias J, Vinas M, Guinea J, Loren JG.
Brown pigmentation in Serratia marcescens cultures associated with tyrosine metabolism. Can J Microbiol 35:1037-1042, 1989.

Tsujii T, Seno S.
Melano-macrophage centers in the aglomerular kidney of the sea horse (teleosts): morphologic studies on its formation and possible function. Anat Rec 226:460-470, 1990.

Wormald R, Foster A.
Clinical and pathological features of chronic glaucoma in north-east Ghana. Eye 4:107-114, 1990.
Abstract : Of 34 consecutive patients with chronic glaucoma seen in north-east Ghana, 22 (65%) were male and seven (21%) were aged under 40 years. Only 17% of eyes had a visual acuity better than 6/18 at presentation. Sixteen of 23 patients who underwent gonioscopy had PAS of which 13 had positive skin snips for onchocerciasis, compared with two out of seven patients with positive skin snips who had open angle glaucoma (p = 0.003). Of 22 trabecular meshworks examined by light microscopy ten (45%) showed marked melanin pigmentation which was more common in younger patients but did not correlate with onchocerciasis infection.

3. MSH, MCH, OTHER HORMONES, DIFFERENTIATION

- Brown DW, Campbell MM, Kinsman RG, White PD, Moss CA, Osguthorpe DJ, Paul PK, Baker BI.
Melanin-concentrating hormone: a structural and conformational study based on synthesis, biological activity, high-field NMR, and molecular modeling techniques. Biopolymers 29:609-622, 1990.
Abstract : A series of Melanin-concentrating hormone (MCH) fragments have been synthesized and their biological activities compared with the parent peptide. The substructural units, 5-14 linear and 5-14 cyclic, have been used as models for MCH-- H-Asp1-Thr-Met-Arg-Cys-Met-Val-Gly-Arg HO-Val17-Glu-Trp-Cys-Pro-Arg-Tyr-Val in 1H-nmr conformational studies. Conformational features predicted by molecular dynamics analyses find support in the nmr experiments.

- Paul PK, Dauber-Osguthorpe P,

Campbell MM, Brown DW, Kinsman RG, Moss C, Osguthorpe DJ.
Accessible conformations of melanin-concentrating hormone: a molecular dynamics approach. Biopolymers 29:623-637, 1990.
Abstract : Molecular dynamics simulations have been used to search for the accessible conformations of the melanin-concentrating hormone (MCH). The studies have been performed on native MCH and two of its peptide fragments, a cyclic MCH(5-14) fragment and a linear MCH(5-14) fragment. An analysis of the molecular dynamics trajectories of the three peptides indicates that two regions of the peptide have characteristic conformational properties that may be important for the biological activity. One is a region around Gly8, which is conformationally mobile, and the other is around Pro13, which shows unusual rigidity. The molecular dynamics simulation results are discussed in terms of backbone structural features like beta turns, side-chain interactions, and orientations of the disulfide bridge. The results of this analysis are used to suggest new analogues that will modify the conformational features of the peptide and further define the conformational requirements for activity. Finally, the results are related to nmr studies of the peptide and reveal agreements between the experimental nuclear Overhauser effect constraints and some of the accessible conformations obtained from the simulation.

4. PHOTOBIOLOGY AND PHOTOCHEMISTRY

- Friedmann PS, Wren FE, Matthews JN.
Ultraviolet stimulated melanogenesis by human melanocytes is augmented by di-acyl glycerol but not TPA. J Cell Physiol 142:334--341, 1990.
Abstract : Epidermal melanocytes (MC) synthesize melanin inresponse to ultraviolet radiation (UVR). The mechanisms mediating the UV-induced activation of melanogenesis are unknown but since UVR induces turnover of membrane phospholipids generating prostaglandins (PGs) and other products, it is possible that one of these might provide the activating signal. We have examined the effects of prostaglandins (PGs) E1, E2, D2, F2 alpha, and di-acyl glycerol upon the UV-induced responses of cultured human MC and the Cloudman S91 melanoma cell line. The PGs had little effect on unirradiated cells and did not alter the response to UVR in either human MC or S91 melanoma cells. However, a synthetic analogue of di-acyl glycerol, 1-oleyl 2-acetyl glycerol (OAG), caused a significant (P less than 0.0001), dose-related augmentation of melanin content both in human MC (seven-fold) and S91 cells (three-fold). UVR caused a significant augmentation of the OAG-induced melanogenesis of both human MC and S91 cells. Since OAG is known to activate protein kinase C, it was possible that the observed modulation of the UVR signal could be via that pathway. Di-octanoyl glycerol, another di-acyl glycerol, which activates kinase C, caused a small (70%) increase in melanogenesis in MC which was not altered by UVR. However, 12-0 tetradecanoyl phorbol 13-acetate (TPA), a potent activator of protein kinase C, had no significant effect on either basal or UV-induced melanin synthesis in either cell type. These data suggest that the UV-induced signal activating melanogenesis could be mediated by di-acyl

glycerol. Furthermore, they imply that the signal is transduced via an alternative, pathway that might be independent of protein kinase C.

- Hirobe T, Zhou XY.
Effects of gamma-radiation on the differentiation of mouse melanocytes in the hair follicles. Mutat Res 234:91-96, 1990.
Abstract : Pregnant mice were whole-body irradiated with a single acute dose of gamma-rays (60Co) to investigate the effect of gamma-radiation on embryonic melanoblasts. The effect was studied by scoring changes in the differentiation of melanocytes in the hair follicles of mice heterozygous for the recessive coat color mutation pink-eyed dilution (p). Abnormal round melanocytes were found in the hair matrix and the dermal papilla of F1 offspring 3.5 days after birth. However, these round melanocytes possessed a melanin deposition of similar intensity to normal hair follicular melanocytes. The frequency of the abnormal hair follicles increased in a dose-dependent manner. Moreover, higher frequencies were found in the animals irradiated at earlier stages of embryonic development. These results indicate that gamma-radiation affects dendritogenesis and the location of mouse melanocytes in the hair follicles, with greater effects seen at the earlier stages of development.

- Leong H, Katz M, Delk A, Nacht S, Berliner D.
Porous polymer beads containing melanin as UV blockers. Eur Pat Appl, 12 pp., 1989.
Abstract : A cosmetic compn. includes melanin incorporated in polymer particles, usually within an internal pore network in the polymer matrix. The melanin may be produced by in situ oxidn. of melanin precursors within the pore network, or by absorption of melanin in a suitable vehicle or carrier. The melanin compn. displays enhanced absorbance of UV radiation and better cosmetic attributes than melanin which is not incorporated in such a polymer matrix. Thus, porous beads of Me methacrylate-ethylene glycol dimethacrylate copolymer were prepd. by adding a soln. of the monomers and benzoyl peroxide in toluene to an aq. phase, stirring to form droplets of org. phase, heating to polymerize, filtering the beads (which had toluene trapped in the pores), and drying to remove the toluene. Melanin was prepd. by oxidn. of tyrosine with persulfate and autopolymn., mixed with 1N NH4OH and L-DOPA, and used to impregnate the dry methacrylate beads. The resulting beads were suspended at 5.3 wt.% in a transparent gelatinous material and a section of the material 0.07-0.09 mm thick was irradiated at 250-290 nm. The UV-blocking ability of the material was 84%, compared to 51% for a 4.8% charcoal suspension.

Stankov B, Lucini V, Snochowski M, Cozzi B, Fumagalli P, Maccarinelli G, Fraschini F.
Cytosolic androgen receptors in the neuroendocrine tissues of the golden hamster: influence of photoperiod and melatonin treatment. Endocrinology (Baltimore) 126:1164, 1990.

5. NEUROMELANINS

- Hirsch EC, Graybiel AM, Agid Y.
Selective vulnerability of pigmented dopaminergic neurons in Parkinson's disease. Acta

Neurol Scand Suppl 126:19-22, 1989.
Abstract : From a neuropathological point, the diagnosis of Parkinson's disease is confirmed by a neuronal cell loss and the presence of Lewy bodies in the substantia nigra. In Parkinson's disease, the precise type of nigral neuron which degenerate still remains unknown. Are all types of neuron similarly injured, are only subpopulations of neurons vulnerable? In an attempt to answer the question, a qualitative and quantitative analysis of the distribution of dopaminergic cells, as identified by immunohistochemistry with a specific antibody against tyrosine hydroxylase, was performed in the ventral mesencephalon of control subjects and patients who died with a clinical diagnosis of Parkinson's disease. In control brains, two types of catecholaminergic neurons were evidenced; some contain visible-neuromelanin, others do not. In patients with Parkinson's disease, the tyrosine hydroxylase positive cells which contained the pigment were the most vulnerable.

- Marchese MJ, McDonald JV.
Intramedullary melanotic schwannoma of the cervical spinal cord: report of a case. Surg Neurol 33:353-355, 1990.
Abstract : We present a case report of a patient with an intramedullary tumor of the midcervical cord. At surgery, the lesion was found to be highly pigmented, and pathological analysis revealed a melanotic schwannoma. Intramedullary schwannomas and melanotic schwannomas are exceedingly rare. This is the second reported case of an intramedullary melanotic schwannoma of the central nervous system.

- Montefiori DC, Modliszewski A, Shaff DI, Zhou J.
Inhibition of human immunodeficiency virus type 1 replication and cytopathicity by synthetic soluble catecholamine melanins in vitro. Biochem Biophys Res Commun 168:200-205, 1990.
Abstract : Synthetic soluble melanins were synthesized by spontaneous oxidation of L-dopamine, norepinephrine or 5-hydroxytryptamine (serotonin) in weak alkaline solution. These three melanins inhibited infection of human CD4+ lymphoblastoid cells (MT-2) by cell-free human immunodeficiency virus type 1 (HIV-1), without cell toxicity, at concentrations of 0.15-10 micrograms/ml. Also, syncytium formation and resulting cytopathic effects when uninfected cells were mixed with chronic HIV-1-infected cells were blocked by these melanins. Antisyncytial activity was greater when infected cells were preincubated with melanin than when uninfected cells were preincubated with melanin, thus suggesting that interaction of melanin with viral proteins is an important aspect of the antiviral mechanism. These results make synthetic soluble melanins interesting candidates for further study as possible anti-HIV-1 therapeutics.

- Ohara S, Kondo K, Kagoshima M, Yanagisawa N.
Secondary degeneration of substantia nigra following massive basal ganglia infarction. Rinsho Shinkeigaku 29:1352-1356, 1989.
Abstract : Two autopsied cases of massive unilateral cerebral infarction due to occlusion of the middle cerebral artery (MCA) were reported with special reference to presence of the secondary degeneration of the substantia nigra. Case 1 was a 70-year old male who suddenly suffered from left hemiplegia 3 years and 2 months prior to death. CT scan showed massive

infarction involving basal ganglia and fronto-parietal white matter on the right side. Some parkinsonian features such as oily face and rigidity of limbs were noted during the course. At autopsy, the proximal portion of rt MCA was found occluded and the right substantia nigra was found depigmented. Case 2 was a 71-year old male who suddenly became hemiplegic 4 years prior to death. CT scan revealed a low density area in the corona radiata of the right cerebral hemisphere. On carotid antiography, complete obstruction of the horizontal portion of right MCA at its distal end was observed, which was confirmed at autopsy. Histologically, the right substant a nigra in case 1 showed marked neuronal loss with gliosis as well as presence of many extracellular melanin pigments. These changes were more prominent in its medial portion where chromatolytic neurons were occasionally seen. The adjacent fronto-pontine tract and pyramidal tract showed secondary degeneration. The left substantia nigra appeared normal. In case 2, the substantia nigra on both sides appeared normal. The whole right cerebral peduncle, on the other hand, showed diffuse myelin pallor.

- Sheng JG.
The effect of prolyl-leucyl-glycine (MIF-1) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on dopamine levels of the striatum. Chung Hua Shen Ching Ching Shen Ko Tsa Chih 22:213-215, 1989.
Abstract : We report that large dose MPTP-treated mice bring about a reduction in the levels of DA, 5-HT and their metabolites in the striatum. In the mice pretreated with PLG, although the striatal DA level was also reduced, mean DA and 5-HT levels were significantly higher than in mice given MPTP alone. It is concluded that PLG can prevent, at least partially, the neurotoxic effect of MPTP. We assume that the mechanism of action of PLG may directly or indirectly reduce MPTP-induced dopaminergic neurotoxicity in the nigrostriatal system or reduce the affinity of MPP+ to melanin in DA cells.

- Youdim MB, Ben-Shachar D, Riederer P.
Is Parkinson's disease a progressive siderosis of substantia nigra resulting in ,iron' and melanin induced neurodegeneration? Acta Neurol Scand Suppl 126:47-54, 1989.
Abstract : Razor sharp and high iron deposits are present in the substantia nigra (SN). Although the function of such high iron content is not known, the homeostasis of brain iron is important for normal brain function. The participation of free tissue iron in oxidative stress (OS), resulting in the formation of cytotoxic hydroxyl radical (.OH) from H2O2 (Fenton reaction) and promotion of membrane lipid peroxides by .OH can no longer be questioned as a biological phenomenon. The highly selective increase of Fe2+ and Fe3+ and lipid peroxidation observed in parkinsonian SN points to OS in such brains. Lipid peroxidation proceeds with either Fe2+ or Fe3+ provided a mechanism exists to facilitate the interconversion of iron between its redox states. Indeed H2O2 derived from MAO B reaction and autooxidation of dopamine to melanin in the SN can drive the iron dependent Fenton reaction. Furthermore, interaction of iron with melanin may be even more important considering that melanin avidly binds Fe3+ and reduce it to Fe2+, resulting in .OH generation. Thus, without evoking environmental neurotoxins, the excessive accumulation of free iron in the SN and "melanin-trap" could be the

trigger for accelerated cell death and Parkinsonism.

6. GENETICS

- Leu WM, Wu SY, Lin JJ, Lo SJ, Lee YH.
Analysis of the promoter region of the melanin locus from Streptomyces antibioticus. Gene 84:267--277, 1989.
Abstract : Several approaches were used to study the transcriptional control region of the melanin-production locus (melC) of Streptomyces antibioticus. Filter-binding in combination with exonuclease III protection localized the 3' boundary of a Streptomyces RNA polymerase-binding site predominantly about 39 nucleotides (nt) upstream from the start codon of melC1, the first open reading frame in the melC locus. Deletion of nt 112-197 upstream from the melC1 start codon reduced melC expression to less than 10%, and deletion of nt 28-107 or 28-120 upstream from melC1 totally inactivated melC. High-resolution nuclease S1 mapping identified the in vitro transcriptional start point (tsp) at 33-34 nt upstream from the start codon of melC1. No sequence resembling the E. coli consensus promoter sequence was found in this region, and site-directed mutagenesis of such a sequence located 101-132 nt upstream from melC1 did not influence melC expression. These studies suggest that transcription of melC is principally from a single tsp and is positively regulated by a mechanism that involves sequences 87-163 nt upstream from the tsp.

- Shibahara S, Okinaga S, Tomita Y, Takeda A, Yamamoto H, Sato M, Takeuchi T.
A point mutation in the tyrosinase gene of BALB/c albino mouse causing the cysteine----serine substitution at position 85. Eur J Biochem 189:455-461, 1990.
Abstract : Murine albinism is characterized by complete lack of melanin pigments in skin and retina. In order to study the molecular basis of albinism, we have cloned and characterized the tyrosinase gene of BALB/c mice (c/c). Sequence analysis of this gene reveals a point mutation at nucleotide residue 387 (G----C transversion) causing a Cys----- Ser substitution at position 85 in one of the cysteine-rich domains of the tyrosinase molecule. Since this G----C transversion creates an additional DdeI site, we were able to confirm that this mutation is actually present in BALB/c genomic DNA using DNA amplification techniques. In contrast, both C57BL/6 (C/C) and DBA/2 (C/C) mouse strains carry the G residue at the same position, suggesting that this point mutation is specific for the albino mutation at the c locus. Moreover, we were able to show that the tyrosinase containing Ser-85 is not functional in transient expression of its cDNA. We therefore suggest that a G----C transversion at nucleotide residue 387 of the tyrosinase gene could lead to the albino phenotype of BALB/c mouse.

- Tanaka S, Yamamoto H, Takeuchi S, Takeuchi T.
Melanization in albino mice transformed by introducing cloned mouse tyrosinase gene. Development (Cambridge, UK) 108:223-227, 1990.
Abstract : A mouse tyrosinase minigene, mg-Tyrs-J, in which the authentic genomic 5'-noncoding

flanking sequence was fused to a mouse tyrosinase cDNA, was introduced into fertilized eggs of albino mice. Of the 25 animals that developed from the injected eggs, 4 mice exhibited pigmented hair and eyes. Histol. anal. of the transgenic mice revealed that the melanogenesis was restricted to hair bulbs and eyes. These results suggest that this minigene encodes active tyrosinase protein and that its 5'-flanking region contains the sequences regulating expression of the mouse tyrosinase gene. This is the first report of successful expression of tyrosinase gene and of pigment prodn. in transgenic mice.

- Tseng HC, Lin CK, Hsu BJ, Leu WM, Lee YH, Chiou SJ, Hu NT, Chen CW.
The melanin operon of Streptomyces antibioticus: expression and use as a marker in gram-negative bacteria. Gene 86:123-128, 1990.

- Vijayasaradhi S, Bouchard B, Houghton AN.
The melanoma antigen gp75 is the human homologue of the mouse b (brown) locus gene product. J Exp Med 171:1375-1380, 1990.
Abstract : The gp75 antigen is an abundant intracellular glycoprotein expressed in melanosomes of human pigmented melanocytes and melanomas. IgG antibodies in sera of a patient with metastatic melanoma have been shown to immunoprecipitate gp75, suggesting that immunological tolerance against gp75 can be broken. The mouse mAb TA99, which specifically recognizes gp75, was used to isolate and purify the antigen. Amino acid sequences of three internal peptides were determined from the purified gp75 polypeptide. cDNA clones were isolated by screening with oligonucleotides based on these peptide sequences. The gp75 peptides and cDNA had approximately 90% identity with, respectively, the derived amino acid and nucleotide sequences of a mouse gene that maps to the b (brown) locus. The brown locus determines coat color in the mouse, suggesting that gp75 regulates or influences the type of melanin synthesized.

- Yamamoto H, Takeuchi S, Kudo T, Sato C, Takeuchi T.
Melanin production in cultured albino melanocytes transfected with mouse tyrosinase cDNA. Idengaku Zasshi 64:121-135, 1989.
Abstract : An attempt was made in the present study to express mouse tyrosinase cDNAs fused with the authentic genomic 5' non-coding flanking sequence in cultured albino melanocytes. One of the cDNA sequences, which expressed successfully and produced melanin pigments, was analyzed with respect to deduced amino acid sequence. Sequencing of the tyrosinase genomic gene revealed the existence of several sets of a characteristic structure which consists of a chain of two successive stem structures, CCAAT-homology and TATA box at its 5'non-coding region. It seems possible that this region represents the regulatory element of the tyrosinase gene. Unusually long GA cluster at 5'upstream region was also found.

7. TYROSINASE AND OTHER ENZYMES

- Kameyama K, Jimenez M, Muller J, Ishida Y, Hearing VJ.
Regulation of mammalian melanogenesis by tyrosinase inhibition. Differentiation 42:28-36, 1989.
Abstract : Melanocyte stimulating hormone (MSH) specifically induces differentiation of mammalian melanocytes. To further define the biochemical events

elicited by this stimulus, we have cloned murine melanoma cells which are either highly responsive or nonresponsive to MSH, and have examined their ultrastructural appearance, their melanogenic activities, and also their expression of tyrosinase. We have found that the basal levels of melanogenic activity in pigmented and nonpigmented cells correlate with expression of surface MSH receptors rather than with production of tyrosinase. Nonpigmented cells produce a potent, highly stable inhibitor of melanogenesis; this inhibitor acts directly on tyrosinase to dramatically and abruptly suppress melanin production. This posttranslational control of tyrosinase activity may represent a critical regulatory point in mammalian pigmentation.

- Kelley SK, Coyne VE, Sledjeski DD, Fuqua WC, Weiner RM.
Identification of a tyrosinase from a periphytic marine bacterium. FEMS Microbiol Lett 67:275--279, 1990.
Abstract : A newly isolated periphytic marine bacterium has been shown to synthesize a true tyrosinase. The enzyme exhibited both cresolase and catecholase functions and catalyzed the biosynthesis of melanin from L-tyrosine. Enzyme activity was enhanced in the presence of oxidants and was inhibited by copper chelating agents such as diethyldithiocarbamic acid and cyanide. The apparent mol. wt. of the 2-40 tyrosinase (67,000) makes this enzyme the largest known prokaryote tyrosinase.

- Kurbanov Kh, Spiridonova NA.
Tyrosine and methionine metabolism at different states of melaninogenesis. Biokhimiya (Moscow) 55:165-172, 1990.
Abstract : Urinary excretion of tyrosine and methionine metabolites as well as the activities of enzymes involved in their metab. are correlated with the state and type of melanin synthesized in the skin of guinea pigs. The response of tyrosine aminotransferase to melaninogenesis induction was more pronounced in animals with predominant pheomelaninogenesis, esp. after tyrosine load, whereas dopachrome oxidoreductase response was increased in animals with predominant eumelaninogenesis and after methionine load. Glutathione reductase and cystathioneine .beta.-synthase responded more vigorously to methionine injections, which was esp. well pronounced in animals with the prominent pheomelaninogenesis and in albino animals. The metabolic block in melanin synthesis in albino animals seem to be obsd. after the 5-S-cysteinyl-DOPA synthesis, whereas the initial steps of melaninogenesis in these animals are identical to pheomelanin synthesis reactions.

- Pawelek JM.
Dopachrome conversion factor functions as an isomerase. Biochem Biophys Res Commun 166:1328-1333, 1990.
Abstract : Dopachrome conversion factor is an enzymatic activity associated with the pigmentary system which catalyzes the conversion of dopachrome, an intermediate in melanin biosynthesis, to dihydroxyindole-2-carboxylic acid (DHICA). To date, the mechanism of action of DCF has been unknown because all previous assays have employed a dopachrome substrate contaminated with L-dopa. It has therefore not been possible to determine whether L-dopa acts as a hydrogen donor in the reaction or whether the formation of DHICA occurs through an isomerization of dopachrome. In this study it is shown that DCF catalyzes the conversion of dopachrome to DHICA equally well in the presence or

absence of L-dopa. The DCF-mediated reaction thus appears to be an isomeric rearrangement of hydrogen ions from one portion of the dopachrome molecule to another. The results indicate that the name "dopachrome isomerase" appropriately describes the function of DCF.

8. MELANOMA

- Ara G, Anderson RR, Mandel KG, Ottesen M, Oseroff AR.
Irradiation of pigmented melanoma cells with high intensity pulsed radiation generates acoustic waves and kills cells. Lasers Surg Med 10:52-59, 1990.
Abstract : Photokilling of pigmented mouse melanoma cells (B-16) was investigated using pulsed high intensity visible radiation. Melanin acts as an endogenous chromophore, and 694 nm radiation with 40 nsec pulse duration and 0.5-3 X 10(7)w/cm2 intensity causes cell death. Irradiation of non-pigmented human melanoma cells (U1) or human squamous carcinoma cells (FaDu) under similar conditions did not kill the cells. Also, irradiation of B-16 cells with 300 microsec laser pulses (10(3)W/cm2) or with continuous wave (CW) radiation (10(-3)W/cm2) did not kill the cells. These data indicate that pigmented cell killing is due to absorption of radiation by melanin and that the pulsewidth and intensity of radiation play important roles in cell killing. The generation of acoustic waves due to absorption of the pulsed radiation by pigmented cells and by isolated melanosomes was demonstrated at 532 and 625 nm and 8.5 nsec pulse duration (10(7)--10(8) W/cm2); the amplitudes of the acoustic signals were approximately 2.5-3.0-fold higher at 532 nm compared with 625 nm, and they increased with increasing fluence. In contrast, irradiation of U1 or FaDu cells with comparable fluences and intensities did not generate acoustic waves. A possible correlation between the generation of photoacoustic waves and pigment cell death is proposed. Since the thermal relaxation time of melanosomes is 0.5-1.0 microsec, the mechanism proposed is that thermal confinement of high intensity, short-pulse visible radiation generates acoustic waves by thermal expansion, leading to mechanical damage to the cells.

- Beerman H, Rigaud C, Bogomoletz WV, Hollander H, Veldhuizen RW.
Melanin production in black medullary thyroid carcinoma (MTC). Histopathology 16:227-233, 1990.
Abstract : Melanin production by two medullary carcinomas of the thyroid is reported and discussed. In both tumours, melanin and calcitonin could be detected in the same cells.

- Carney JA.
Psammomatous melanotic schwannoma. A distinctive, heritable tumor with special associations, including cardiac myxoma and the Cushing syndrome. Am J Surg Pathol 14:206-222, 1990.

- Coderre JA, Halle DA.
Direct electrophilic iodination of 2-thiouracil using Iodo-Gen. Int J Rad Appl Instrum [A] 40:759-763, 1989.
Abstract : 5-Iodo-2-thiouracil (ITU) is of interest due to its ability to bind specifically to the pigment melanin during melanogenesis and is of potential value in the diagnosis and treatment of malignant melanoma.

Radioiodinated ITU was prepared directly from 2-thiouracil in a two-phase reaction using Iodo-Gen in 0.05 M phosphate buffer pH 7.0. The identity radiochemical purity and stability of the product were checked by reversed-phase high pressure liquid chromatography (HPLC). ITU labeled with 123I, 125I or 131I has been produced in millicurie amounts and isolated on a semi-preparative reversed-phase HPLC column. Production time was 2-3 h, overall radiochemical yields averaged 80%; the radiochemical purity was greater than 98%. Specific activities on the order of 20 Ci/mmol have been obtained.

- Coma-del-Corral MJ, Perez-Serrano L, Razquin-Lizarraga S.
Melanotic adenocarcinoma of the anorectum. J Clin Gastroenterol 12:114-117, 1990.

- Hazuka MB, Edwards-Prasad J, Newman F, Kinzie JJ, Prasad KN.
Beta-carotene induces morphological differentiation and decreases adenylate cyclase activity in melanoma cells in culture. J Am Coll Nutr 9:143-149, 1990.
Abstract : Several studies suggest that beta-carotene reduces the risk of some cancers. Except for its function as an antioxidant, the effect of this vitamin on mammalian cells remains poorly defined. This study was performed to show whether beta-carotene treatment of murine B-16 melanoma cells in culture induces differentiation and alters the adenylate cyclase (AC) system. The AC system mediates the action of agents which regulate cell differentiation and transformation. Results showed that beta-carotene treatment for a period of 24 hours or more caused morphological differentiation without changing the level of melanin, and reduced basal and melanocyte-stimulated hormone (MSH)-, sodium fluoride (NaF)-, and forskolin-stimulated AC activity in vitro. Retinol, a metabolite of beta-carotene, inhibited growth without morphological differentiation and reduced basal and MSH- and NaF-stimulated AC activity. However, butylated hydroxyanisole, a lipid-soluble antioxidant, also reduced growth without morphological differentiation, but it failed to alter basal or MSH-stimulated AC activity. The present and previous studies show that the AC system represents a common site where some antitumor-promoting vitamins (beta-carotene, retinol, retinoic acid, and alpha-tocopheryl succinate) act.

Inoue S, Ito S, Wakamatsu K, Jimbow K, Fujita K.
Mechanism of growth inhibition of melanoma cells by 4-S-cysteaminylphenol and its analogues. Biochem Pharmacol 39:1077-1083, 1990.
Abstract : Our previous studies have shown that 4-S-cysteaminylphenol (4-S-CAP) causes a significant inhibition of in vivo melanoma growth and a marked depigmentation of black skin and hair follicles. These studies have suggested a role of tyrosinase in the manifestation of these in vivo effects. In this study 4-S-CAP and its analogues were examined for their effects on the growth of human melanoma cells in vitro. 4-S-CAP and 4-S-HomoCAP exhibited strong cytotoxicity with effects much greater than those of alpha-methyl-4-S-CAP and N,N-dimethyl-4-S-CAP. The cytotoxicity of the former two amines was completely prevented by semicarbazide, an inhibitor of plasma monoamine oxidase, while that of the latter two was not prevented by semicarbazide, catalase, and phenylthiourea, a tyrosinase inhibitor. In culture medium 4-S-CAP was rapidly converted by the action of monoamine oxidase present in fetal bovine serum to the

aldehyde which was then metabolized to the alcohol and the carboxylic acid when cells were present. alpha-Methyl-4-S-CAP was found to exert higher cytotoxicity to cells with higher tyrosinase activity and melanin content. These results suggest that the in vitro cytotoxicity of 4-S-CAP and 4-S-HomoCAP is mediated through conversion to the aldehydes while that of alpha-methyl-4-S-CAP appears to be dependent on tyrosinase activity to some extent.

- Kuchel O, Buu NT, Edwards DJ.
  Alternative catecholamine pathways after tyrosine hydroxylase inhibition in malignant pheochromocytoma. J Lab Clin Med 115:449--453, 1990.

- Langer K, Konrad K.
  Congenital melanocytic nevi with halo phenomenon: report of two cases and a review of the literature. J Dermatol Surg Oncol 16:377-380, 1990.

- Mair TS, Pearson GR.
  Melanotic hamartoma of the hind brain in a riding horse. J Comp Pathol 102:239-243, 1990.

- Yonemoto K, Kondoh S, Nishiyama S.
  Characteristics of gamma glutamyl transferase in melanoma. Nippon Hifuka Gakkai Zasshi 99:1139--1144, 1989.
  Abstract : Gamma glutamyl transferase (GGT) is widely distributed in mammalian tissues, and its biological heterogeneity has been demonstrated among various tissues. In order to investigate the biological characteristics of GGT in melanomas, enzyme-histochemical and biochemical studies were performed using amelanotic/-melanotic and murine/human melanomas as materials. Enzyme-histochemically, GGT activity appeared to be present only in melanogenic cells in vitro. Biochemical assays of tissue extracts revealed that the specific activity was much higher in melanotic melanomas than in amelanotic. In addition, analysis of GGT-isoenzymes demonstrated that an isoenzyme band at approximately 110KD was expressed in tumorigenic or highly-metastatic tissues. These findings suggest that GGT in melanoma is closely related to the ability of melanin production and that the possible existence of a unique isoenzyme may reflect the intensity of tumorigenic and/or metastasis.

9. EYE

- Cabral L, Unger W, Boulton M, Lightfoot R, McKechnie N, Grierson I, Marshall J.
  Regional distribution of lysosomal enzymes in the canine retinal pigment epithelium. Invest Ophthalmol Vis Sci 31:670-676, 1990.

- Chijiiwa T, Ishibashi T, Inomata H.
  Histological study of choroidal melanocytes in animals with tapetum lucidum cellulosum. Graefes Arch Clin Exp Ophthalmol 228:161-168, 1990.

- Dearry A, Edelman JL, Miller S, Burnside B.
  Dopamine induces light-adaptive retinomotor movements in bullfrog cones via D2 receptors and in retinal pigment epithelium via D1 receptors. J Neurochem 54:1367--1378, 1990.

- Dorey CK, Torres X, Swart T.
  Evidence of melanogenesis in porcine retinal pigment epithelial cells in vitro. Exp Eye Res

50:1-10, 1990.
Abstract : Several weeks after porcine retinal pigment epithelial (RPE) cell cultures attain confluence, macroscopically visible brown foci appear. The cuboidal cells that form the foci contain numerous phase dark granules that do not exhibit the autofluorescence characteristic of lipofuscin. The data described here indicate that the granules are melanosomes. Electron microscopy revealed three types of electron-dense granules in these cells: simple spheres 0.3-0.5 microns in diameter, large spheres 1-2 microns in diameter, and lysosomal aggregations of the smaller spheres. The matrix of both spheres is composed of 40-nm microvesicles that were also found free in the cytoplasm and aggregated within vacuolar structures. Reversed-phase high-performance liquid chromatography of RPE cells and their media detected melanogens, i.e. intermediates of melanin biosynthesis, including several indole derivatives. The porcine RPE cultures therefore may be a useful system for studying melanogenic regulation.

- Fite KV, Bengston L.
Aging and sex-related changes in the outer retina of Japanese quail. Curr Eye Res 8:1039-1048, 1989.

- Lee KP, Valentine R.
Retinotoxicity of 1,4,-bis(4-aminophenoxy)-2-phenylbenzene (2-phenyl-APB-144) in albino and pigmented rats. Arch Toxicol 64:135-142, 1990.
Abstract : Albino and pigmented strains of rats were administered 0, 5, 25, 100, or 500 mg/kg 2-phenyl-APB-144 by gavage and were killed 14 days later. Although no ocular lesions were found in rats dosed at 5 mg/kg, similar dose-related retinopathy was found at 25, 100, or 500 mg/kg in both albino and pigmented rats. The primary target site appeared to be retinal pigment epithelial (RPE) cells and photoreceptor outer segments (POS). At 25 mg/kg or greater, multifocal retinal detachment with disrupted POS occurred where the RPE cells showed necrotic changes and contact loss with POS due to fragmentation of apical processes in the RPE cells. Also, RPE cells showed hyperplasia, migration, and phagocytic activity toward disrupted POS. The photoreceptor nuclei (outer nuclear cells) were displaced into the areas occupied by disrupted POS. At 100 or 500 mg/kg, multifocal or diffuse disruption of POS and photoreceptor inner segments (PIS) was obsd. with markedly proliferating RPE cells. The photoreceptor nuclei were disorganized, less numerous, and necrotic. Some photoreceptor nuclei directly apposed the RPE cells or Bruch's membrane due to the absence of both POS and PIS. The cytoplasm of RPE cells was loaded with phagosomes, disrupted lamellar disks, myelin bodies, and lysosomal residual bodies. The morphol. changes appeared to be related to lysosomal dysfunction of the RPE cells. The presence of melanin pigment in the RPE cells did not appear to be the primary factor in the development of the retinopathy.

- Menon IA, Trope GE, Basu PK, Wakeham DC, Persad SD.
Binding of timolol to iris-ciliary body and melanin: an in vitro model for assessing the kinetics and efficacy of long-acting antiglaucoma drugs. J Ocul Pharmacol 5:313-324, 1989.
Abstract : Topical beta blockers are used to treat glaucoma patients. These drugs inhibit aqueous production for prolonged periods of time. The purpose of this study was to determine whether timolol maleate (a non-specific beta blocker) binds to

human iris-ciliary body (CB) melanin and to elucidate the binding characteristics of the drug to melanin. Timolol bound to bovine iris and ciliary body by two possible mechanisms. The binding kinetics indicate that the binding is probably of a nonspecific nature. There was no statistically significant differences between the melanotic tissues (CB, iris) and the nonmelanotic tissues (lens, cornea, liver, kidney) regarding the amount of timolol bound. However significantly more timolol was bound to the isolated melanins than the whole tissues. Timolol was released from the nonmelanotic tissues at a much faster rate than from the melanotic tissues. The amount of timolol bound to iris-CB from albino and pigmented rabbits showed that the amount of timolol bound to these tissues diminished in the following order: black or gray greater than brown greater than albino. It was also found that the rate of timolol release decreased in the following order: albino greater than gray greater than brown or black. Our results demonstrate the binding of beta blocker to human, bovine and rabbit iris-CB and consequent slow release of timolol from these tissues.

- Mircheff AK, Miller SS, Farber DB, Bradley ME, O'Day WT, Bok D. Isolation and provisional identification of plasma membrane populations from cultured human retinal pigment epithelium. Invest Ophthalmol Vis Sci 31:863-878, 1990.

Abstract : We have attempted to isolate samples of apical and basal-lateral plasma membranes from cultured fetal human RPE. Cells from confluent, dome-forming cultures were disrupted with a Dounce apparatus. Nuclei and melanin granules were sedimented by centrifugation at 2600 g for 10 min. The supernates were layered over gradients of 17.5-65% sorbitol and centrifuged at 122,000 g for 5 hr. Fractions were grouped into "density windows" on the basis of their biochemical marker contents. Na,K-ATPase and alkaline phosphatase overlapped but did not precisely parallel one another, suggesting associations with two partially separated membrane populations; in density window I, alkaline phosphatase was enriched 4.3-fold, and Na,K-ATPase was enriched 1.7-fold, whereas in window II the corresponding enrichment factors were 7.7 and 6.7. These markers were well resolved from a mitochondrial marker, but they were overlapped by endoplasmic reticulum and Golgi markers. Additional density gradient centrifugations, performed after samples had been suspended in 55% sorbitol, further separated alkaline phosphatase- and Na,K-ATPase-containing membranes from endoplasmic reticulum and Golgi membranes, yielding alkaline phosphatase and Na,K-ATPase cumulative enrichment factors of 6.8 and 2.5 for the sample from window I and 9.3 and 10.9 for the sample from window II. Subsequent phase partitioning analysis of the sample from window I further enriched an alkaline-phosphatase-rich membrane population, which is believed to represent the RPE basal-lateral membranes. The sample from density window II contained two membrane populations, both enriched in Na,K-ATPase, alkaline phosphatase, and galactosyltransferase, and both of which appear to be derived from the apical plasma membrane. SDS-PAGE and Western blotting confirmed a correlation between Na,K-ATPase catalytic activity and Na,K-ATPase alpha subunit immunoreactivity.

- Sakina NL, Dontsov AE, Afanas'ev GG, Ostrovski MA, Pelevina II. The accumulation of lipid

peroxidation products in the eye structures of mice under whole-body x-ray irradiation. Radiobiologiia 30:28-31, 1990.
Abstract : In studying the effect of whole-body X-irradiation on the accumulation of lipid peroxidation products (conjugated dienes, TBA-active products, and Schiff bases) in retina and retinal pigmented epithelium of pigmented and nonpigmented mice it was shown that irradiation of dark-pigmented mice does not cause even a slight accumulation of lipid peroxidation products as compared to that in the controls. Albino mice exhibited a marked increase in the level of lipid peroxidation products which was manifested soon after irradiation and persisted for at least 3 months after irradiation. Melanine is suggested to participate in protecting eye structures against pro-oxidizing action of ionizing radiation.

10. OTHER

- Aver'yanov AA, Lapikova VP, Petelina GG, Dzhavakhiya VG.
Increased sensitivity of pigment mutants of Piricularia oryzae to toxic excretions of rice leaves. Fiziol Rast (Moscow) 36:1088-1095, 1989.
Abstract : The effects of spores of a melanin-contg. pathogenic strain of the fungus P. oryzae and its melanin-deficient non-pathogenic alb-1 and ros-1 mutants on the generation of superoxide radical by rice leaves and on superoxide-mediated fungitoxicity of leaf diffusates were studied. The mutants, in contrast to the wild type, did not enhance the toxicity of leaf diffusates, but were more sensitive to it. The melanin from the wild-type strain, when added in excess to spores, dramatically diminished their injury both in diffusates and in a Fenton-reaction model system generating oxygen radicals. Prepns. isolated by the same method from the mutants were much weaker protectors than melanin. Apparently, the non-pathogenicity of the strains may be related to their enhanced sensitivity to leaf excretions. It may be a consequence of the absence, deficiency, or structural disturbances of spore melanin which neutralizes the oxygen radicals generated by leaves.

- Fleming MG, Bergfeld WF.
A simple immunochemical technique for distinguishing melanocytes and melanophages in paraffin-embedded tissue. J Cutan Pathol 17:77-81, 1990.
Abstract : We describe an immunoperoxidase technique for distinguishing melanocytes and melanophages in paraffin-embedded tissue. Two primary antibodies were used: anti-S100 and HAM-56, a monoclonal antibody directed against macrophages. In a variety of pigmented lesions, HAM-56 labelled melanophages while S100 labelled cells of melanocytic lineage.

- Naughton GK, Jacob L, Naughton BA.
A physiological skin model for in vitro toxicity studies. Altern Methods Toxicol 7:183-189, 1989.
Abstract : A three-dimensional skin model is being utilized as a substrate for a variety of toxicity assays. This histol. complete model is established by seeding dermal fibroblasts onto a nylon mesh which has been pretreated to optimize cell adherence. When suspended in liq. medium, the fibroblasts form a dermal equiv. by stretching across the 210 .mu.m openings and depositing collagens and other

matrix proteins. By 5-7 d a full-thickness dermis with mitotically and metabolically active fibroblasts is established. Cocultured melanocytes and keratinocytes continue to produce melanin when inoculated onto this dermis and remain above a naturally formed dermal/epidermal junction. Keratinocytes exhibit normal differentiation patterns and secrete keratin proteins. Human cells grown with this skin model have been used successfully as substrates for cytotoxicity and skin penetration studies. This culture system is easily adaptable to std. cytotoxicity assays and can be used as a tool to study the effect of a variety of compds. on normal neonatal and adult skin in vitro.

- Wilkins AL, Day ME, Rowland ML. Hair-darkening kits containing metal salts and organic ligands. Eur Pat Appl, 12 pp., 1989.

****

NEWS FROM THE ESPCR

June 1990 - Updated ESPCR membership list

Prof Allegri
Dept Pharmaceutical Sciences
University of Padova
Via Mazzolo 5
35100 Padova
ITALY

Dr F Amilcarelli
Dept of Cell Physiology
University of L'Aquila
Via Assergi 6
67100 L'Aquila
ITALY

Prof F Anders
Genetisches Institut
Justus Liebig Universität
Heinrich-Buff-Ring 58-62
6300 Giessen
F.R.G.

Dr L Andreassi
Clinical Dermatologica
Università di Siena
Piazza Duomo 2
53100 Siena
ITALY

Dr R Aquaron
Lab de Biochimie Médicale
Faculté de Médecine
27 Bd Jean Moulin
13385 Marseilles Cedex 5
FRANCE

Dr K Arstrand
Dept Clinical Chemistry
University Hospital
581 85 Linkoping
SWEDEN

Dr C Aubert
Lab de Recherches sur les Cancers Cutanés et la Pigmentation
INSERM U. 119
27 Bd Leï Roure
13009 Marseilles
FRANCE

Dr A Augustsson
Dept Dermatol./Venereol.
Sahlegrens Hospital
Karl-Gustavsgatan 36
411 31 Gothenburg
SWEDEN

Prof T Bagnara
Dept of Anatomy
Arizona Health Sciences Center
1501 N Campbell Avenue
Tucson, Arizona 85724
USA

Dr M-L Barrenas
Dept Occupational Noise
Röda Stråket 12
Sahlgrenska Sjn
413 45 Gothenburg
SWEDEN

Dr R-N Bartelt
Praunheimerweg 25
6 Frankfurt/Main 50
F.R.G.

Dr M Benathan
Dept of Dermatology
CHUV
1011 Lausanne
SWITZERLAND

Dr D Bennett
Dept of Anatomy
St George's Hospital
Medical School
Cranner Terrace
London SW17 0RE
UK

Dr O Berthier-Vergnes
INSERM U 218
Centre Léon Bérard
28 rue Laënnec
69373 Lyon Cedex 08
FRANCE

Dr M Biasolo
Dipto di Scienze Farmaceutiche
Università di Padova
Via Mazzolo 5
35100 Padova
ITALY

Dr M Birch
Biology & Metastasis Laboratory
Imperial Cancer Research Fund
Lincoln's Inn Fields
London WC2A 3PX
UK

Prof S Bleehen
Dept of Dermatology
Royal Hallamshire Hospital
Sheffield S10 2JF
UK

Dr J Borovansky
Dept of Biochemistry
Charles University
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr D Botti
Dept Biology & Cell Physiology
University of L'Aquila
Via Assergi 6
67100 L'Aquila
ITALY

Mr M Boué-Grabot
BIOGIR S.A.
Z.I. de Toctoucau
33610 Cestas
FRANCE

Prof R Bowers
Dept of Biology
California State University
5151 State Univ Drive Center
Los Angeles CA 90032
USA

Prof A Breathnach
Division of Anatomy, UMDS
St Thomas's Campus
St Thomas Hospital Med School
Lambeth Palace Road
London SE1 7EH - UK

Dr M Bridelli
Dept of Physics
University of Parma
Via delle Scienze
43100 Parma
ITALY

Dr G Brooks
Biology & Metastasis Lab
Imperial Cancer Research Fund
Lincoln's Inn Fiels
London WC2A 3PX
UK

Dr C Brufau
Avenue Juan Carlos I,6,7H
30 008 Murcia
SPAIN

Dr G Calderini
FIDIA s.p.a.
Via Ponte d. Fabbrica 3/A
35031 Abano Terme (PD)
ITALY

Dr N Cascinelli
Ist. Nazionale dei Tumori
Via Venezian 1
20133 Milano
ITALY

Dr C Causse
L'Oréal
1 Av. Eugène Schueller
93600 Aulnay-Sous-Bois
FRANCE

Dr J-P Césarini
LRTPH Fondation Rotschild
INSERM
25 rue Manin
75019 Paris
FRANCE

Dr R Cicero
Ist. di Biologia Generale
Policlinico
Piazza G. Cesare
70124 Bari
ITALY

Mr C Cooksey
Dept of Chemistry
University College London
Gower Street
London WC1H 0AJ
UK

Dr O Crescenzi
Dept Organ. & Biol Chem.
University of Naples
Mezzocannone 16
80134 Napoli
ITALY

Prof P Crippa
Dipto di Fisica
Università di Parma
Via delle Scienze
43100 Parma
ITALY

Dr Del Porto
Cattedra di Genetica Medica
c/o Ospedale L. Spallanzani
Via Portuense 292
00149 Roma
ITALY

Dr A Deflandre
L'Oréal
1 Av. Eugène Schueller
93600 Aulnay-Sous-Bois
FRANCE

Dr De Wolff-Rouendaal
Dept of Ophthalmology
Academic Hospital
PO Box 9600
2300 RC Leiden
THE NETHERLANDS

Dr J-F Doré
INSERM U 218
Centre Léon Bérard
28 rue Laënnec
69373 Lyon Cedex 08
FRANCE

Dr G Duchon
Dept of Biochemistry
Charles University
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr M Dumas
LVMH Recherches
48-50 Rue de Seine
92703 Colombes Cedex
FRANCE

Dr A Eberle
Dept of Research (ZLF)
University Hospital
Hebelstr.20
4031 Basel
SWITZERLAND

Mr J Edwardson
3 Elm Bank Close
Leamington Spa
Warwickshire CV32 6LR
UK

Prof M Eisner
Dept of Physics
University of Houston
4000 Calhoun Road
Houston, Texas 77004
USA

Dr C Ferrer
Dept of Cell Biology
School of Medicine
30100 Murcia
SPAIN

Dr P Forlot
Laboratoires Goupil S.A.
30 Av. Du Président Wilson
94320 Cachan
FRANCE

Dr A Fourtanier
L'Oréal
1 Av Eugène Schueller
93600 Aulnay-Sous-Bois
FRANCE

Prof E Frenk
Dept of Dermatology
University of Lausanne
1011 Lausanne
SWITZERLAND

Dr P Friedmann
Dept of Dermatology
Royal Victoria Infirmary
Newcastle upon Tyne NE1 4LP
UK

Prof P Fritsch
Dept of Dermatology
University of Innsbrück
Anichstr. 35
6020 Innsbrück
AUSTRIA

Prof Dr D Gabel
Universität Bremen
Fachbereich Chemie
Postfach 330 440
2800 Bremen 33
F.R.G.

Dr A Gallone
Ist. Biologia Generale
Policlinico
Piazza G. Cesare
70124 Bari
ITALY

Dr Y Gauthier
75 Cours de Luze
33300 Bordeaux
FRANCE

Dr E Geremia
Ist. di Biologia Generale
Via Androne 81
95124 Catania
ITALY

Dr G Ghanem
Lab. Oncology & Exp Surgery
Institut Jules Bordet
1 Rue Heger-Bordet
1000 Brussels
BELGIUM

Dr P Giacomoni
L'Oréal
1 Av. Eugène Schueller
93600 Aulnay-sous-Bois
FRANCE

Dr P Grammatico
Cattedra di Genetica Medica
Università di Roma "La Sapienza"
c/o Ospedale Lazzaro Spallanzani
Via Portuense 292
00149 Roma
ITALY

Prof B Guarneri
Clinica Dermatologia
Policlinico
Universitario Gazzi
98100 Messina
ITALY

Dr M Hadley
Dept of Anatomy
Arizona Health Sciences Center
University of Arizona
Tucson, Arizona 85724
USA

Dr C Hansson
Dept of Dermatology
University Hospital
221 85 Lund
SWEDEN

Dr E D Hearing
Data Abstract Search Serv.
2477 Regina Drive
Clarksburg, MD 20871
USA

Dr V Hearing
National Institute of Health
Bldg 37, Rm 1B22
Laboratory of Cell Biology
Bethesda, MD 20892
USA

Dr A Hedin
Dept of Periodontology
Centrallasarettet
631 88 Eskilstuna
SWEDEN

Dr H Z Hill
New Jersey Medical School
MSB-E586
185 South Orange Avenue
Newark, NJ 07103-2757
USA

Prof A Holl
Inst. Gal. & Special Zool.
Justus-Liebig Universität
Heinrich-Buff-Ring 38/MZVG
6300 Giessen
F.R.G.

Dr J E Hornby
Dept of Pure & Applied Zoology
University of Reading
Reading RG1 5DP
UK

Prof J A Hunter
Dept of Dermatology
Level 4, Phase 1 Building
The Royal Infirmary
Edingburgh EH3 9YW
UK

Dr M d'Ischia
Dept Organic & Biol Chemistry
University of Naples
Via Mezzocannone 16
80134 Naples
ITALY

Dr D N Joseph
Stiefel Laboratories
Wooburn Green
High Wycombe
Bucks HP10 0AU
UK

Dr B Kagedal
Dept of Clinical Chemistry
University Hospital
581 85 Linkoping
SWEDEN

Dr M Kemali
Ist di Cibernetica del C.N.R.
Via Toiana 6
80072 Arco Felice
Naples
ITALY

Dr S Klaus
Dept of Dermatology
Hadassah Hospital
PO Box 12000
91-120 Jerusalem
ISRAEL

Dr E M Kokoschka
II Dermatology Dept
University of Vienna
Alserstr. 4
1090 Vienna
AUSTRIA

Dr N Kollias
Physics Dept
Kuwait University
Safat 133060
KUWAIT

Prof B Lagerlöf
Dept of Pathology
Karolinska Hospital
104 01 Stockholm
SWEDEN

Dr E J Land
Paterson Laboratories
Christie Hospital
& Holt Radium Institute
Wilmslow Road
Manchester M20 9BX
UK

Dr G Lang
L'Oréal
1 Av. Eugène Schueller
93600 Aulnay-Sous-Bois
FRANCE

Dr B S Larsson
Dept of Toxicology
Uppsala University
Box 594
751 24 Uppsala
SWEDEN

Prof F J Lejeune
Lab. Oncology & Exp Surgery
Institut Jules Bordet
1 Rue Heger-Bordet
1000 Brussels
BELGIUM

Dr L Lenner
Dept of Clinical Chemistry
Regionsjukhuset
58 185 Linkoping
SWEDEN

Prof K Lerch
Biochemisches Institut
University of Zurich
Winterhurerstr. 190
8057 Zurich
SWITZERLAND

Dr N Lindquist
Dept of Toxicology
University of Uppsala
Box 594
751 22 Uppsala
SWEDEN

Dr E M Link
Dept Chemical Pathology
University College &
Middlesex School of Medicine
Windeyer Bldg, Cleveland St
London W1P 6DB
UK

Dr J Lofberg
Dept of Zoology
Uppsala University
Box 561
751 22 Uppsala
SWEDEN

Prof J A Lozano
Inst. de Biochimica Clinica
Conjunto Residencial
Apartado 61
Murcia
SPAIN

Prof R MacKie
Dept of Dermatology
Glasgow University
Glasgow G12 8QQ
UK

Dr I Maida
Ist Di Biologia Generale
Facultà di Medicina
Piazza di G. Cesare
70124 Bari
ITALY

Dr A Mallardi
Ist di Biologia Generale
Facultà di Medicina
Piazza di G. Cesare
70124 Bari
ITALY

Dr E Mansson-Brahme
Radiumhemmet
Karolinska Hospital
104 01 Stockholm
SWEDEN

Prof C D Marsden, F.R.S.
Dept of Neurology
Institute of Psychiatry
De Crispigni Park
London SE5
UK

Prof J M Mascaro
Dept of Dermatology
Hospital Clin. y Provincial
c/o Casanova 143
08036 Barcelona
SPAIN

Dr B Matous
Dept Med Chemistry & Biochem
Faculty of General Medicine
Charles University
U nemocnice 5
128 53 Prague 2
CZECHOSLOVAKIA

Dr H Menke
St Franciscus Gasthuis
Kleiweg 500
3045 PM Rotterdam
THE NETHERLANDS

Dr A Meyer zum Gottesberge
Dept of Otorhynology
University of Düsseldorf
Moorenstr. 5
4000 Düsseldorf
F.R.G.

Prof F L Meyskens
University of California
Irvine Medical Center
101 City Drive South Orange
California 92668-3298
USA

Dr M Miranda
Dept Biology & Cell Physiology
Università de L'Aquila
Via Assergi 6
67100 L'Aquila
ITALY

Prof Y Mishima
Dept of Dermatology
Kobe Univ. School of Med.
5-1 Kusunoki-cho
7-Chome Chuo-Ku
Kobe
JAPAN

Prof G Misuraca
Dept Pharmacology
Faculty of Pharmacy
University of Naples
Via Montesano
80100 Naples
ITALY

Dr G Monfrecola
Clinica Dermatologica II
Facoltà di Medicina
Università di Napoli
Via Pansini 5
80131 Napoli
ITALY

Dr A Napolitano
Dept Organic & Biol Chem
University of Naples
Via Mezzocannone 16
80134 Napoli
ITALY

Dr M Nazzarro-Porro
Ist Dermatologica
San Gallicano
Via San Gallicano 25/A
00153 Roma
ITALY

Prof R A Nicolaus
Dept of Chemistry
University of Naples
Via Mezzocannone 16
80134 Naples
ITALY

Dr H I Nielsen
Medi-Cult a/s
Kanalholmen 12
2650 Hvidovre
DENMARK

Dr P Y Noël
Lab of Reproductive Physiology
Université P. & M. Curie
7 Quay St Bernard
75252 Paris Cedex 05
FRANCE

Dr M Ochotorena
Dept of Pathology
Faculty of Medicine
University of Murcia
Espinardo
30100 Murcia
SPAIN

Dr K D O'Hare
Dept of Biological Science
Keele University
Staffordshire ST5 5BG
UK

Dr K Olander – NIHS
Dept of Toxicology
Uppsala University
Biomedicum
Box 594
75 124 Uppsala
SWEDEN

Dr I Onsman
Emmakade 52
1102 AR Amstelveen
THE NETHERLANDS

Prof Dr Med. C E Orfanos
Dept of Dermatology
Univ. Med Centre Steglitz
The Free Univ. of Berlin
Hindenburgdamm 30
1000 Berlin 45 (West)
F.R.G.

Prof J P Ortonne
Service de Dermatology
Hôpital Pasteur
BP 69
30 Av. de la Voie Romaine
06100 Nice Cedex
FRANCE

Dr A Palumbo
Stazione Zoologica
Villa Communale
80121 Napoli
ITALY

Dr P Parsons
Queensland Institute for
Medical Research
Herston
Queensland
AUSTRALIA

Dr S Passi
Ist Dermatologico S Gallicano
Via San Gallicano 25
00153 Roma
ITALY

Dr S Pavel
Dept of Dermatology
University of Amsterdam
Academisch Medisch Centrum
Meibergdreef 9
1105 AZ Amsterdam
THE NETHERLANDS

Dr R U Peter
Dept of Radiobiology
Germ Armed Forces Med Acad
Neuherbergstr. 11
8000 Munich 45
F.R.G

Dr A Petterson
Dept Clinical Chemistry
University Hospital
581 85 Linkoping
SWEDEN

Dr M Picardo
Ist Dermatologico S. Gallicano
Via San Gallicano 25/A
00153 Roma
ITALY

Dr G Pintucci
Ist di Biologia Generale
Facoltà di Med. e Chirurgia
Policlinico
Piazza G. Cesare
70124 Bari
ITALY

Dr A Poma
Dept Cell Biol & Physiology
University of L'Aquila
Via Assergi 6
67100 L'Aquila
ITALY

Prof G Prota
Dept Organic & Biol Chemistry
University of Naples
Via Mezzocannone 16
80134 Naples
ITALY

Dr M Prunieras
72 Avenue Mozart
75016 Paris
FRANCE

Dr A Ragnelli
Dept Biol & Physiol Cell
University of L'Aquila
Via Assergi 6
67100 L'Aquila
ITALY

Dr A Ramaiah
Dept of Biochemistry
All India Institute of Medical
Sciences
New Dehli 110029
INDIA

Dr M Ranson
33 Fernsdale Street
Newton 2042
AUSTRALIA

Dr A Rastrelli
FIDIA s.p.a.
Via Ponte della Fabbrica 3/A
35031 Abano Terme (PD)
ITALY

Dr C Renieri
Ist Produzioni Animali
Via S. Costanzo 4
06100 Perugia
ITALY

Prof P A Riley
Dept Chemical Pathology
University College &
Middlesex School of Med
Windeyer Bldg, Cleveland St
London W1P 6DB
UK

Dr B Rindone
Dept Organic & Ind. Chemistry
University of Milano
Via Venezian 21
20133 Milano
ITALY

Dr U Ringborg
General Dept of Oncology
Karolinska Hospital
104 01 Stockholm
SWEDEN

Dr A Roberto
Dept of Toxicology
Uppsala University
Box 594
571 24 Uppsala
SWEDEN

Prof H Rorsman
Dept of Dermatology
Lasarettet
221 85 Lund
SWEDEN

Dr I Rosdahl
Hudpolikliniken
Lundby sjukhus
Wieselgrensplatsen 19
417 17 Göteborg
SWEDEN

Prof Y Salomon
Dept of Hormone Research
The Weizmann Institute of
Science
PO Box 26
Rehovot 76100
ISRAEL

Dr C Santoro
Ist de Biologia Generale
Via Androne 81
95124 Catania
ITALY

Prof T Sarna
Inst of Molecular Biology
Jagiellonian University
Al Mickiewicza 3
31 120 Krakow
POLAND

Dr M Scalia
Ist di Biologia Generale
Via Androne 81
95124 Catania
ITALY

Prof D Schachtschabel
Inst für Physiologische Chemie
Philipps Universität Lahnberge
3550 Marburg
F.R.G.

Dr K U Schallreuter
Dept of Dermatology
University of Hamburg
Martinistr. 52
2000 Hamburg 20
F.R.G.

Dr M Schartl
Genzentrum
Max-Planck-Inst für Biochem
Am Klopferspitz 18
8033 Martinsried
F.R.G.

Dr A Schothorst
Dept of Dermatology
University Hospital Leiden
Bldg 26, PO Box 9600
2300 RC Leiden
THE NETHERLANDS

Dr P Schrier
Dept Clinical Oncology
University Hospital
Bldg I, K1-P
PO Box 9600
2300 RC Leiden
THE NETHERLANDS

Dr A Schrott
Dept of ENT
Anichstr. 35
6020 Innsbrück
AUSTRIA

Prof F Serri
Director, School of
Specialization in Dermatology
Catholic Univ of the S. Heart
Largo A. Gemelli 8
00168 Roma
ITALY

Prof G Sichel
Ist di Biologia Generale
Via Androne 81
95124 Catania
ITALY

Dr N Smit
Dept of Dermatology
University of Amsterdam
Academisch Medisch Centrum
Meibergdreef 9
1105 AZ Amsterdam
THE NETHERLANDS

Dr F Solano-Munoz
Dept of Biochemistry
Faculty of Medicine
University of Murcia
Espinardo
30100 Murcia
SPAIN

Dr U Stierner
Dept of Oncology
University of Gothenburg
Sahlgren's Hospital
413 45 Gothenburg
SWEDEN

Dr T Tallberg
Helsinki Univ Central Hosp.
Laboratory of Immunology
Mannerheimintie 172
00280 Helsinki 28
FINLAND

Miss A J Taylor
Sir William Dunn School of Pathology
University of Oxford
South Parks Road
Oxford OX1 3RE
UK

Dr A J Thody
Dept of Dermatology
Univ. of Newcastle upon Tyne
Royal Victoria Infirmary
Newcastle upon Tyne NE1 4LP
UK

Mr D Thome
Muehlstr. 31
6300 Giessen
F.R.G.

Dr A Vercammen-Grandjean
Laboratory of Oncology
& Experimental Surgery
Free University of Brussels
1 Rue Heger-Bordet
1000 Brussels
BELGIUM

Dr M Vetterlein
Institute for Tumorbiology
& Cancer Research
University of Vienna
Borschkegasse 8A
1090 Vienna
AUSTRIA

Dr O Vicente
Dept of Pathology
University of Murcia
Espinardo
30100 Murcia
SPAIN

Prof B Weatherhead
Dept of Anatomy
Faculty of Medicine
Li Shi Fan Building
5 Sassoon Road
HONG KONG

Prof A Weinreb
Racah Institute of Physics
Dept of Physics
Givat Ram
Hebrew University of Jerusalem
91904 Jerusalem
ISRAEL

Dr W Westerhof
Dept of Dermatology (F7Z)
Academic Medical Centre
Meibergdreef 9
1105 AZ Amsterdam
THE NETHERLANDS

Dr I D Williams
Rm 106 Materials Research Lab
Pennsylvania State University
University Park
PA 16802
USA

Dr L J Wolfram
c/o Clairol Inc.
2 Blackley Road
Stamford, CONN 06902
USA

Prof H Wyler
Inst de Chimie Organique
Université de Lausanne
2 Rue de la Barre
1005 Lausanne
SWITZERLAND

Dr K Yamada
Memanbetsu-cho
Abashiri-gun
Hokkaido
JAPAN

Dr A Zuasti
Depto Biologia Celular
Facultad de Medicina
Universidad de Murcia
30100 Murcia
SPAIN

****

Received in Brussels

PIGMENT CELL RESEARCH BULLETIN

NAME : .....

ADDRESS : .....

.....

PHONE : .....

CONTRIBUTIONS

I shall send my contribution in the form of :

COMMENTARY

DISCUSSION

REVIEW

LETTER TO THE EDITOR

TOPIC COVERED : .....

NUMBER OF PAGES : .... (not exceeding 6 pages in double spacing, including references)

NB : when available, please send it on a diskette (MS-DOS) along with a copy of your text.

ANNOUNCEMENTS

I wish to announce the following :

MEETING

COURSE

RESEARCH OPPORTUNITY

OTHER

* : please mark

Please send back to :

G. GHANEM, PhD
Laboratory of Oncology and Experimental Surgery
Institut Jules Bordet
Rue Héger-Bordet 1
B - 1000 Bruxelles

Minutes of the EORTC Melanoma Cooperative Group

EORTC European Organization for Research and Treatment of Cancer

EORTC European Organization for Research and Treatment of Cancer

MINUTES

MELANOMA COOPERATIVE GROUP
Chairman: U.R. Kleeberg (Hamburg)
Secretary: J.F. Doré (Lyon)
Treasurer: S. Carrel (Epalinges)

Spring Meeting 1990: April 26 – 28th.
Tellerup Park Hotel
Copenhagen, Denmark

Host:
Prof. K.T. Drzewiecki and the Danish Melanoma Group
Department of Plastic Surgery
Rigshospitalet, Copenhagen

Participants:

G. Andry, BRUXELLES, M.F. Avril/VILLEJUIF, M. Bergenmar/STOCKHOLM, Y. Brandberg/STOCKHOLM, B. Bröcker/MUNSTER, L. Brosetti/FIRENZE, J.P. Cesarini/PARIS, C. Chartier/STRASBOURG, P. Chemaly/PARIS, N. Crespeigne/BRUXELLES, B. Czarnetzki/BERLIN, P. de Wit/NIJMEGEN, J.F. Doré/LYON, K.T. Drzewiecki/COPENHAGEN, F. Enderlin/St GALLEN, E. Engel/HAMBURG, J.D. Freitas/COIMBRA, A. Galli/FIRENZE, W. Gatzemeier/GÖTTINGEN, B. Gerard/BRUXELLES, N. Haas/BERLIN, N. Hastrup/COPENHAGEN, S. Henzen – Logmans/ROTTERDAM, N.K. Jensen/ODENSE, J.P. Johnson/MÜNCHEN, A. Junker/MINDEN, H. Kjeldsen/AARHUS, U.R. Kleeberg/HAMBURG, K. Kölmel/GÖTTINGEN, B. Kroo/AMSTERDAM, F.J. Lejeune/BRUXELLES, J. Lock – Andersen/KRISTIANSTAD, H. Luther/BOCHUM, A. McKay/GLASGOW, R. MacKie/GLASGOW, E. Månsson Brahme/STOCKHOLM, S. Moretti/FIRENZE, N. Neumann/HANNOVER, A. Østerlind/COPENHAGEN, H. Panisen/ODENSE, N.C. Petersen/AARHUS, E. Pichler/INNSBRUCK, U. Ringborg/STOCKHOLM, D.J. Ruiter/NIJMEGEN, R. Rümke/AMSTERDAM, E. Scheffer/AMSTERDAM, H. Schraffordt – Koops/GRONINGEN, P.J. Schner/LEIDEN, S. Suciu/BRUXELLES, L. Suter/MUNSTER, D. Thomas/BRUXELLES, M. Thorn/UPPSALA, F. Truchetet/THIONVILLE, M. Vaglini/MILANO, R. Versteeg/HAMBURG, C. Vilmer/St CLOUD, H. Voigt/KALTENKIRCHEN.

Workshop on the epidemiology of skin melanoma
(in association with the Danish Melanoma Group and the Danish Cancer Society)

- Olsen G (Copenhagen).
  Introduction and welcome
- Magnus K (Oslo).
  Descriptive epidemiology of malignant melanoma of the skin in the five nordic countries.
- Østerlind A (Copenhagen).
  The Danish case – control study of malignant melanoma of the skin: constitutional variables and sun exposure.
- English D (Lyon).
  The epidemiology of common acquired naevi and their relationship to malignant melanoma of the skin.
- Drzewiecki KT (Copenhagen).
  Dysplastic naevi and their relationship to malignant melanoma of the skin.
- Hastrup N (Copenhagen).
  The incidence of histopathologically verified dysplastic naevi in contiguity with malignant melanoma in Denmark.
- Drzewiecki (Copenhagen).
  Trends in prognostic factors for primary malignant melanoma in Denmark 1949 – 1988.
- Thörn M (Stockholm).
  Trends in survival of malignant melanoma of the skin in Sweden.
- Ringborg U (Stockholm).
  Screening strategies for malignant melanoma of the skin and its precursors.
- MacKie RM (Glasgow).
  Can we prevent melanoma developing through health education?

Workshop on the treatment of skin melanoma localized to head and neck. – Free papers.

- Lefebvre JL (Lille)
  Head and neck melanoma of the skin. Current trials of the EORTC head and neck tumors group.
- Ringborg U (Stockholm)
  Head and neck melanoma of the skin – results of treatment in Sweden.
- Andersson A and Gottlieb J (Copenhagen)
  Head and neck melanoma of the skin – results of treatment in Denmark.
- Andry G (Brussels).
  Wide surgery and reconstruction for head and neck melanoma.
- Drzewiecki KT (Copenhagen)
  Surgical treatment of melanoma of the skin of the head and neck. One cm versus two cm excision. Protocol of the Scandinavian Melanoma Study Group.
- Dahlstrøm K (Copenhagen).
  Problems with management of giant naevi in children.
- Gjedde S (Copenhagen)
  Tumour infiltrating lymphocytes (TIL) in malignant melanoma. Experimental and clinical background.
- Zeuthen J (Copenhagen).
  TIL – immunological studies. Experience and results of Copenhagen Study Group.
- Gundersen HJG (Århus)
  Stereological methods for malignancy grading of tumours.
- Brandt Sørensen F (Århus)
  Stereological estimation of nuclear volume for malignancy grading of malignant melanoma.

Ongoing research in the member institutions

- Kölmel KF (Göttingen).
  Treatment of metastatic melanoma by endotoxin – A phase I study.
- de Wit P (Nijmegen).
  Expression of the epidermal growth factor receptor in human melanocytic lesions.
- Versteeg R (Hamburg).
  Influence of cytotoxic drugs, GM – CSF and antiestrogens on proliferation rate and the antigenic phenotype of human melanoma cell cultures.
- Liénard D and Lejeune FJ (Brussels).
  Isolation perfusion with rTNF alpha, rIFN gamma and Melphalan.
- Schrier P (Leiden).
  ras oncogene activation in human melanoma.

Clinical trials – interim report

– *Report of the Data Center on Phase – III trials* (D. Thomas and S. Suciu)

(N.B. – The Data Center report is available from the Secretary, upon request, for active members only)

(18781): Long term immunotherapy comparing two BCG – preparations for stage I high risk melanoma.
Study coordinator: B. Czarnetzki (Berlin)
Date of activation June 21, 1979, trial closed January 1987, the objective being to compare the duration of the disease free interval and survival in patients treated with BCG Pasteur vs. BCG RIV vs. control after curative surgery. 342 eligible patients. 60% of the patients have been analysed as to their BCG reactivity. Final analysis expected this year after a median follow – up of 5 years.

(18832): A randomized trial on prophylactic isolation perfusion for stage I high risk melanoma of the limbs.
Study coordinators: M. Vaglini (Milano), F. Lejeune (Bruxelles), E. Krementz (USA)
Date of activation March 1, 1984. So far 566 patients entered the trial from 16 institutions. Much emphasis was given to various aspects of quality control: Histopathological slide review c/o Dr. Clemente, Milano, for the "Southerners" and Dr. Ruiter, Nijmegen, for the "Northerners". Quality control and site – visits have markedly improved the surgical techniques of the participating institutions. Coordinator Dr. H. Schraffordt – Koops.
Since, aside from the effect of IP, a number of important prognostic indicators are being examined, it was proposed at the Verona meeting (October, 1989) that this trial is to continue until about 1000 patients have been included. This decision was confirmed by the majority of participants.
Data on late side effects (all patients after one year) will be added to the forms to be distributed.

EORTC
European Organization for Research and Treatment of Cancer

(18871 (DK 80–1)) adjuvant trial in melanoma comparing Interferon r Alpha2 to r Gamma (to Iscador – only protocol DK 80–1) and to a control group after surgical removal of either high risk primary (>3mm) or curative resection of lymph node metastasis.
Study coordinator: U.R. Kleeberg (Hamburg)
Date of activation January 1988. Participating centers must decide to which trial they want to participate (i.e EORTC without or EORTC–DK with Iscador). This decision cannot be changed. Sofar 18 institutions have opted for the EORTC protocol and have entered 84 patients and 10 institutions have opted for the EORTC–DK protocol and have entered 108 patients, i.e. a total of 192 patients.
Testing of anti–gamma/IFN should be discontinued. The first recurrence should be biopsied.
More centers are still invited to participate.

– *Report on ongoing Phase–II trials* (U.R. Kleeberg)

(18852): Pilot Phase–II study on recombinant Interferon alpha2 in advanced melanoma.
Study coordinators: F. Lejeune (Bruxelles), M. Prade (Villejuif), S. Carrel (Epalinges)
Date of activation July 22, 1986. This trial has been closed to patient entry. 41 patients have entered the trial. The main interest of this phase–II study concerns the immunological alterations of the tumor cell phenotypes due to r IFN alpha2 treatment. First results have been published during the 2nd International Melanoma Conference in Venice (E. Bröcker et al.) showing a modulation of progression markers.

(18881): Phase–II study of Fotemustine in patients with advanced melanoma.
Study coordinators: U.R. Kleberg (Hamburg), S.P. Israels (Amsterdam)
Trial activated in 1988, closed in april 1990. 140 patients have been entered. The results are being analysed and will be published shortly.

(18891): Phase–II study of Toremifene in patients with advanced melanoma.
Study coordinator: U.R. Kleeberg (Hamburg)
Trial activated in 1989. Sofar 27 patients entered. This trial, testing a new antiestrogen and its effect on cytokines both in vivo and in vitro, supplemented by immunological side studies as to the tumor cell phenotype is open to patient entry. Interested clinicians as well as basic scientists are invited to contact the Secretary.

Quality control

A panel of surgical oncologist, chaired by H. Schraffordt–Koops (Groningen) as well as pathologists, chaired by D. Ruiter (Nijmegen) and clinicians, chaired by U.R. Kleeberg (Hamburg), are following both phase–II as well as phase–III studies to improve clinical technology, histological as well as immunohistochemical and clinical diagnosis and treatment. The work of these extramural review committees is considered to be of particular importance for the quality of clinical research. In addition, the EORTC quality control committee is offering site–visits to study the validity of data collection and evaluation (Mrs. N. Crespeigne, Bruxelles).

Cellular biology – Immunology subgroup (S. Carrel, Epalinges and P. Schrier, Leiden)

A study of *n–ras* oncogene activation in melanoma is being initiated, with two aims: specificity of codon 13 mutation, and role of exposure to UV in the activation of *n–ras* oncogene. Participants are invited to send tumors, LM as well as SSM, and eventually tumors arising in Xeroderma patients (ideally new paraffin blocks), with the following particulars : localization of the primary, skin type, sun behavior (if available) to Dr. P. Schrier, Dept. of Clinical Oncology, University Hospital Leiden, Building I–K_1–P, P.O. Box 9600, 2300 RC Leiden, Tel. (0031) 71–261916.
Anyone, both in the MCG and the ESPCR, interested in this project is invited to contact either Dr. P. Schrier or the Secretary.

Pathology subgroup (D.J. Ruiter, Nijmegen)

The subgroup is currently working on the histopathological criteria of dysplastic naevi. 50 cases were circulated to observers during a workshop.

Melanoma epidemiology (F. Lejeune, Bruxelles)

In cooperation with the SEARCH Program of the International Agency for Research on Cancer in Lyon (Dr. P. Boyle), two epidemiological studies are currently being set up: a cohort study of patients with Dysplastic Naevus Syndrome, and a case–control study of the role of sun exposure in melanoma development in Europe.

EORTC
European Organization for Research and Treatment of Cancer

UICC TNM

UICC is preparing a revision of the TNM classification of malignant melanoma. Suggestions from our members are welcome and should be directed to the Chairman.

Cooperation with Eastern Europe

MCG is highly desiring to establish cooperation with Eastern Europe. If you know of colleagues who could join our activities, please contact them and drop a notice to the Chairman. Grants may be available for such cooperations.

EORTC Oncology Nursing Group (ONG)

Dr. Don Newling, coordinator of the clinical groups initiated the idea, supported by the board of the EORTC, to establish a nursing group within the EORTC. Miss Clementine Molin has been nominated as head of the new ONG. Two aims are envisaged: 1) to allow the specialist oncology nurses to meet their colleagues from other cooperative groups and other countries to discuss common problems in the management of patients with cancer, 2) to look at the possibility of involving representative senior nurses from major institutions in the group's activities and maybe even in the twice yearly meetings.
Having these specialist oncology nurses involved in planing and persuing our clinical trials would certainly greatly improve the work of our clinical teams. So, if you are interested please try to identify nurses with whom the EORTC–ONG could make an initial contact. Secretary and Chairman of the MCG will help to realize this new venture.

Epilogue

The members of the Melanoma Cooperative Group gratefully acknowledge the warm hospitality and the perfect organization of our danish hosts, Pr. K.T. Drzewiecki and the Danish Melanoma Group. They offered three days of excellent scientific exchange and an impressive view of the danish way of life.

Future meetings

Our next meeting will be held in Hamburg, in connection with the 15th International UICC Cancer Congress (August 17th–22nd, 1990), c/o Pr. Dr. U.R. Kleeberg, H.O.P.A., Max Brauer Allee 52, D–2000 Hamburg 50, Tel. (0049)–40–38021232, Fax. 38021215, on Thursday, August 16th, 1990, 14.00 – 19.00 (p.m.), at the *Evangelisches Zentrum Rissen, Iserbarg 1, D–2000 HAMBURG–RISSEN (Tel. 0049 – 40 – 81902120)*. It will start with the plenary session, open to all our members.
This meeting will be preceded by workshops, restricted to registered active members only, on Wednesday, August 15th, 11.00 a.m. (sharp) at the *Museumshafen Övelgönne, Anleger Neumühlen, D–2000 HAMBURG–ALTONA*.
The third part of our meeting will consist of a Symposium on the Immunobiology of melanomas, jointly organized with the UICC Cancer Congress, on Friday, August 17th, 8.30 – 11.30 (a.m.), at the *Hörsaal A, University of Hamburg, Edmund Siemers Allee*.

The 1991 Spring meeting will be held in Innsbruck, April 26–27th 1991, c/o Dr. E. Pichler, Universitäts Hautklinik Anichstr. 35, A–6020 Innsbruck, and will include a Consensus meeting on Melanoma education and prevention (Pr. R. Macfie).
The 1991 Fall meeting will be held in Amsterdam, September 6-7, 1991, prior to the 3rd ESPC Meeting (September 8–11th, 1991), c/o Dr. B.B.R. Kroon, Department of Surgery, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam.

Hoping to see you all soon,
With best regards,
Yours

U.R. Kleeberg (Chairman)
Hamburg

J.F. Doré (Secretary)
Lyon

ANNOUNCEMENTS

RELATED ACTIVITIES

Dear Reader,

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

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

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

Thank you in advance for your cooperation.

G. Prota
President

SURVEY OF CURRENT PIGMENT CELL RESEARCH - 1990

1. FIELD OF STUDY :

2. TITLE / DESCRIPTION :

3. INVESTIGATOR(S) :

4. INSTITUTION :

5. ADDRESS :

6. TELEPHONE :

7. FAX :

8. COMMENTS :

9. TOPIC :

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

A N N O U N C E M E N T

The Division of Dermatology and Cutaneous Sciences at the University of Alberta is pleased to announce that it will host the Third Meeting of the Panamerican Society for Pigment Cell Research on

July 11 - 13, 1991

at

Bernard Snell Hall
University of Alberta
Edmonton, Alberta, Canada

July 11 - Registration and Keynote Speakers

July 12 - Plenary Session, Workshop, Posters

July 13 - Symposium on Malignant Melanoma

The program includes four keynote speakers, workshops, symposium, plenary, and poster sessions, and exhibits.

Topics to be addressed included, among other subjects, Growth Factors and Regulators for Melanocytes and Melanoma, Molecular Biology of UV Phototoxicity, Melanoma Precursors, as well as contributed papers to be chosen at a later date.

The ***call for abstracts*** will be mailed in November, 1990, and travel stipends will be available for graduate students fellows and junior faculty members.

Send inquiries to:

Yolande Matsusaki
General Secretary of the Third Meeting
Conference Centre
#4 Lister Hall
University of Alberta
Edmonton, Alberta
T6G 2H6

Tel. 403-492-7200
Fax. 403-492-7032

Kowichi Jimbow, Ph.D., M.D.
Chairman of the Third Meeting
Division of Dermatology and
Cutaneous Sciences
Faculty of Medicine
260G Clinical Research Wing
Heritage Medical Research Centre
University of Alberta
Edmonton, Alberta T6G 2S2

Tel. 403-492-2425

The Brighton International Conference

MELANOMA '91

May 8–11 th 1991

A major multidisciplinary conference to address problems of the natural history, diagnosis, management and prevention of melanoma

DERMATOLOGY

ONCOLOGY

PATHOLOGY

SURGERY

Keynote Adresses
Multidisciplinary Symposia
Proferred Papers
Biopsy Seminars
Workshops

In association with the Melanoma Study Group

Further details from :
Dr N. Kirkham
Histopathology
Royal Sussex County Hospital
Brighton, BN2 5BE, UK
Tel : 0273 696955 ex. 4269
Fax : 0273 600182