ABSTRACTS OF THE VII INTERNATIONAL PIGMENT CELL CONFERENCE

ENERGY METABOLISM OF MOUSE MELANOMA. K. ADACHI, S. KONDO, M. HIRAGA, F. HU AND M. BELL (Oregon Regional Primate Research Center, Beaverton, Ore. 97005)

Some aspects of the energy metabolism of B16 mouse melanoma, both melanotic and amelanotic, are reported. By means of isotopically labeled glucose, we calculated the contribution of the Embden-Meyerhof pathway, the pentose cycle, the TCA cycle, etc. to the utilization of glucose and the net synthesis of ATP (chemical form of energy). We have also isolated intact mitochondria from mouse melanoma. They show a respiratory control ratio of 2 to 3 and an ADP/O ratio of 2 with succinate. Antimycin A completely inhibits respiration. The mitochondria contain all members of the electron transport system, and phosphorylation is tightly coupled with oxidation. Data thus far obtained indicate that in mouse melanoma the enhanced glycolysis, as in other types of malignant tumors, contributes significantly to the energy formation; however, considerable amounts of ATP are still produced through the TCA cycle. We have found no evidence of respiratory derangement in mouse melanoma. We are currently studying the control steps in these energy metabolism pathways.

CELLS IN THE HUMAN SKIN, OTHER THAN MELANOCYTES, WHICH TAKE UP L-DOPA. JACK ADAMS-RAY (Surgical Clinic, Karolinska sjukhuset, Stockholm, Sweden)

 Autoradiography with tritium-labelled L-dopa has shown other cells than the melanocytes in the human skin taking up and concentrating L-dopa. They do not seem to be mast cells, some of which in other animal species take up L-dopa, transforming it into dopamine. They are so numerous, that we do not think they belong to the group of true chromaffin cells, with the same ultrastructure as the cells in the suprarenal medulla which have been found in human skin. Long-wave ultra violet light does not only cause a direct pigmentation. It also causes a change in the texture of the skin, denoting a release presumably of catecholamines. The above-mentioned cells after long-wave U.V. increase their uptake of L-dopa. Erythrocytes take up dopa, dopamine and noradrenaline both in vivo and in vitro.


The ultrastructure of 4 spindle-B ocular melanomas was examined. Explant cultures of these tumors were established and cells maintained for 6 to 24 months in serial passage. Two of the tumors were deeply pigmented grossly and 2 were lightly pigmented. The tumors were composed mostly of closely-packed rather uniform cells. The 2 heavily pigmented tumors contained cells with large numbers of melanosomes and a lesser number of premelanosome. These melanoma cells appeared similar to normal choroidal melanocytes. The lightly pigmented tumors varied with regard to the presence of melanin precursors. In 1, numerous premelanosome were present in most cells. In the other most cells showed no premelanosome. All tumors contained a variable number of macrophages. No virus particles were seen. In tissue culture, a rapid decrease in the number of premelanosome and melanosome was observed. By 12 weeks (4 passages) in culture no evidence of premelanosome or melanosome was found, although compound melanin granules were plentiful. A similar cessation of pigment formation was noted in tissue cultures of normal choroid and pigment epithelium. Under the same conditions a control culture of nonocular melanoma cells, however, continued to produce pigment for 12 months.

DIFFERENTIATION OF THE MELANOPHORE, IRIDOPHORE, AND XANTHOPHORE FROM A COMMON STEM CELL. NANCY J. ALEXANDER (Oregon Regional Primate Research Center, Beaverton, Ore. 97005)

There are 3 highly stratified layers of chromatophores in the dermis of the adult African high-casuied chameleon, Chamaeleo hoehnelli. The most superficial layer consists of xanthophores. Xanthophore include membranes-bound and generally have been shown to contain carotenoids and pteridines. Subjacent to this layer are the iridophores, which are packed with birefringent rodlets essentially composed of guanine. The basal chromatophores are melanin-containing melanophores with dendritic extensions that terminate above the xanthophores at the dermo-epidermal junction.

In the young chameleon, the 3 layers of chromatophores are less organized but still quite recognizable. In addition to the characteristic pigment inclusions of the xanthophores and iridophores, premelanosome-like inclusions are found distributed in these 2 cell types. Thus, at this time of development, these chromatophores are not complete differentiated. The melanophores are very similar to those of the adult, containing many melanin granules plus some premelanosome. A fourth cell type characteristic of the young chameleon is named the "chromatoblast." The chromatoblast contains many premelanosome-like inclusions, but no mature melanin granules. It is postulated that after the presumptive pigment cells migrate from the neural crest, they become chromatoblasts (stem cells). The melanocytes then begin to differentiate into one of the three chromatophores found in the adult, dependent on their position in the dermis.

GENETIC ASPECTS OF MALIGNANT MELANOMA. DAVID E. ANDERSON (University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025)

Malignant melanoma in man has long been a neoplasm of unknown etiology. However, evidence is beginning to accumulate indicating the existence of a genetic variety. Presently under investigation are 31 families containing from 2 to 16 verified cases of melanoma per family. The familial patients are characterized by a significantly earlier age at first diagnosis and a higher frequency of multiple primaries than that generally reported for melanoma. Although the familial distribution patterns are consistent with a genetic etiology, the genetic data are not compatible with single gene segregation patterns. Sex-linkage is excluded by similar affection rates between males and females and the occurrence of father to son transmissions. Conceivably, the underlying genetic mechanism may involve at least two gene loci. Melanomas are known to develop from pre-existing junctional nevi and particularly in light complected individuals. Since both features are inherited, it is interesting to speculate that these features acting jointly may determine an individual's genetic susceptibility to the development of melanoma. Some preliminary evidence suggests that the genetic variety of melanoma has a higher survival rate than the sporadic variety (5-year survival of 60% vs 47%, respectively).

INTERRELATIONSHIPS OF MELANOPHORES, IRIDO­PHORES AND XANTHOPHORES. JOSEPH T. BAGNARA (University of Arizona, Tucson, Ariz. 85721)

The pigment-containing organelles of melanophores, iridophores, and xanthophores of cold-blooded vertebrates have been designated respectively, melanosomes, reflecting platelets and pterinosomes. The pigments of melanosomes are melamins, those of reflecting platelets are purines, and those of pterinosomes are pteridines. Melanosomes and pterinosomes are approximately 500 μ in diameter as are vesicular shaped
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**HISTOCHEMICAL AND ELEMENTAL STUDIES OF NEUROMELANIN AND LIPOFUSCIN. Herbert Barden and Eugene Martin (Columbia University, New York, N.Y. 10032 and Philips Electronic Corp., Mt. Vernon, N.Y. 10550)**

Neuromelanin in the rhesus monkey and human substantia nigra and lipofuscin in the rhesin inferior olive were found to have interrelated properties. Neuromelanin was naturally non-autofluorescent in UV light and refractile in the dark field. After bleaching with 10% hydrogen peroxide, neuromelanin became autofluorescent and non-refractile. These changes were sufficiently gradual that the inverse relationship of auto-
fluorescent and refractile properties was unequivocally observed in the large intraneuronal aggregates of human neu-
romelanin, Lipofuscin exhibited the properties of bleached neuromelanin. In the inferior olive, but not in the substantia nigra, lipofuscin also demonstrated weak ferrous ion binding capacity. If this metal binding capacity was utilized, olivary lipofuscin could be invested with ferrous sulfide and pseudo-
peroxidatively melanized by dopa. Melanized lipofuscin exhibited the properties of unbleached neuromelanin. Neu-
romelanin invested with ferrous or cupric sulfide could be melanized with dopa or dopamine. Electron probe analysis of neuromelanin, lipofuscin, and neuprol revealed a sulfur content greatest in neuromelanin and least in neuprol. Phos-
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**DEMONSTRATION OF DENDRITIC BRIDGES BETWEEN MIGRANT LIMBAL MELANOCYTES IN THE CORNEA OF THE GUINEA PIG. Robert S. Bart and Alfred W. Kopp (New York University School of Medicine, New York, N.Y. 10016)**

Limbial melanocytes migrate centrally into the cornea when colchicine solution is repeatedly applied to the eye of the living guinea pig. Specimens of such a cornea with its immigrant melanocytes can be maintained for many hours in a modified Sykes-Moore chamber which is perfused continu-
ously with nutrient medium. The transparent quality of this medium allows the observation of melanocytes and other cell types. Examples: melanophages of the red-banned salamander contain perinexasomes, iridophores of the dove iris contain melanosomes, melanophores of a frog's eye contain reflecting plates, xanthophores of a garter snake contain reflecting plates. It is suggested that in the differentiation of chromo-
matoblasts there is present a primordial organellle which under appropriate developmental cues can form either melanosomes, reflecting plates or pterinosomes forming respectively mela-

**ACTIVATION OF MELANOMA ADENYL CYCLASE BY MSH. Mark W. Bitesnky, Harry B. Demopoulos (New York University School of Medicine, New York, N.Y. 10016)**

In 1964, MSH was a hormone without a defined cellular receptor and thus a member of a select group whose number had dwindled as a consequence of studies demonstrating the role of adeny cyclase in hormone action. In addition to guilt by association, there was circumstantial evidence to support the activation that MSH worked via adeny cyclase. This included the observation that methyl xanthines (which prevent enzymic breakdown of cyclic AMP) caused melanosome dis-
ersion in frogs; that ACTH (which shares a 7 amino acid sequence with MSH) was a cyclase activator in adrenal and mimicked MSH effects in amphibians and mammals; and the demonstration that cyclic AMP could darken frog skin in vitro. The case was incomplete, however, since direct activation of melanocyte cyclase by MSH had not been observed. There existed the problem of obtaining a homogenous melano-
cyte source. Frog skin and hamster melanoma were found un-
suitable. However, melanomas from fish and mice (both melanotic and melanotic types) exhibited adeny cyclase ac-
tivity which responded in vitro, selectively, and vigorously to MSH and ACTH. The mechanism whereby an increase in intracellular cyclic AMP causes melanosome dispersion (in amphibians) and an increase in melanin synthesis (in mammals) remains obscure.

**CUTANEOUS DEPIGMEN TATION BY CHEMICAL AGENTS. S. S. Blehean (University College Hospital Medi-
cal School, London, W. C. 1, England)**

A selective destructive effect of certain chemical compounds on guinea pig melanocytes has been observed. A number of substituted phenols have been tested for their depigmenting effect by daily application in concentrations on the wax-injected skin of the back and the unepilated skin of the ear of black guinea pigs. 4-Isopropylcatechol (4IPC) was found to be the most effective depigmenting compound of those tested and in low concentrations did not irritate the skin. In areas treated with 5 G % 4IPC, a uniform depigmen-
tation occurred in 1 to 2 weeks, and with 3 G % and 1 G % 4IPC in 2 to 4 weeks. Depigmentation occurred only in the treated areas and the pigmentation of the hair was unaffected. In the depigmented areas there was a marked reduction in the population of melanocytes (from 600-800 to 0-50 melanocytes per mm²). Many of the remaining melanocytes had lost their dendrites and were only weakly dopa-positive. Electron microscopy revealed few melanocytes and these contained imperfectly melanised melanosomes. The keratinocytes were mainly devoid of melanised melanosomes, but otherwise were unaffected. The Langerhans cells appeared normal. 4IPC was

**ULTRASTRUCTURE OF MELANOMA GRANULES IN THE SKIN OF SUBHUMAN PRIMATES. Mary Bell (Oregon Regional Primate Research Center, Beaverton, Ore. 97005)**

The distribution of melanocytes has been documented in a wide variety of primates with light microscopy (Machida and Perkins, 1967, In: Adv. in Biol. of Skin, Vol. 8, The Pig-
mentary System, ed. by W. Montagna and F. Hu, Pergamon Press, Oxford). The present report is a study of the ultra-
structure of pigment granules in scalp skin from nineteen species, including heavily pigmented primates, such as, e.g., howler monkeys; moderately pigmented species, e.g., marmosets; and virtually non-pigmented species, e.g., galagos.

In heavily pigmented species, melanocytes in all stages of melanogenesis are abundant in the epidermis and its appen-
dages. Adjacent keratinocytes contain numerous melano-
somes, many of which occur in membrane-bound clusters. Melanocytes are less commonly seen in moderately pigmented species; the melanosomes occur predominantly as individual granules, rarely as clusters. In virtually non-pigmented skin, melanocytes are rarely identifiable, and a scant population of individual melanosomes can be found in keratinocytes.

Dermal melanocytes are present in many species and contain abundant melanosomes; these are usually seen as individual granules but also occur as membrane-bound clusters. Pre-
melanosomes, however, are rare in these cells.
found to be an effective depigmenting agent, acting selectively on melanocytes and leading to their destruction and disappearance.

**DIAGNOSTIC USE OF I-131 IODOQUINE IN MELANOMA.** MARSDEN S. BLOIS (Stanford University School of Medicine, Stanford, Calif. 94305).

Visible changes in pigmented lesions of the cutaneous surface are readily noted by the patient, members of his family, or the physician; however, the hidden internal metastases of malignant melanomas, like those of any cancer, are difficult to detect and are of great prognostic importance. Unlike other cancers, the pigmented melanoma by virtue of its pigimentary system and the associated biochemical pathways, offers several approaches to the problem of occult tumor detection.

We will report on the clinical use of a radioactively tagged compound (Iodoquine) which preferentially binds to melanin pigment and by means of which occult melanoma metastases have been localized. This technique has now been used with 18 melanoma patients with the following results: positive results were obtained with 7 patients, with independent confirmation, and with 3 patients without independent confirmation as yet. Negative results were found in 7 patients, all of whom are apparently free of disease, and one false negative test occurred.

**CATECHOLAMINES IN THE SKIN OF AMPHIBIANS IN RELATION WITH THE PHYSIOLOGICAL MELANOPHORE REACTION.** E. BROUWER (Zoological Laboratory, University of Utrecht, The Netherlands)

The action of MSH in the pigment dispersion of the melanophores in amphibians is generally supposed to be indirect. Biogenic amines may be involved in this hormone regulated process. In *Xenopus laevis* a catecholamine has been identified, localized in a strip just below the epidermis. Chemical analysis of skin extracts showed the catecholamine to be dopamine. The relation between dopamine in the skin and the dispersion reaction was investigated in different ways: (1) The quantity of histochemically identifiable catecholamine stored in the skin of black background adapted animals is far less than in white background adapted ones. (2) With methyl-p-tyrosine (-MPT), an enzyme inhibitor of tyrosine hydroxylase, the synthesis of dopamine can be blocked. When black background adapted animals were injected with -MPT, dispersed melanophores gradually aggregate. Chemical analysis indicates an obvious decrease of dopamine in the skin of these animals. (3) Electron microscopic analysis revealed whorl-like structures restricted to those areas of back skin, where dopamine has been localized before. Moreover, some structural differences of the whorls were observed in black and white background adapted animals.

**DEVELOPMENTAL ANALYSIS OF THE SPECULUM OF RANA PIPIENS.** LEON BROUDER (University of Minnesota, Minneapolis, Minn. 55455. Present address: University of Colorado, Boulder, Colo. 80302).

A reaction in the leopard frog (*Rana pipiens*) has been described that produces a reduction in the numbers of pigmented xanthophores and iridophores, the yellow and iridescent pigment cells respectively. The mutant gene, specule, is incompletely dominant and variably expressed. The mode of action of the mutant gene was studied utilizing the techniques of experimental embryology. To determine whether the effects of the mutant gene are hormonally mediated, anterior-posterior chimeras were constructed of mutant anterior ends and wild-type posterior ends. The resulting chimeric pigment patterns demonstrate that no hormonal or diffusible substance from either the mutant or wild-type half affects the differentiation of iridophores and xanthophores of the other half. Transplants of prospective pigment cells of the neural crest from mutant donors to wild-type hosts and vice-versa differentiated according to donor genotype, indicating that the speckle gene expresses its effects in the pigment cells themselves and not through extrinsic means. (Supported in part by grants from the American Cancer Society, NIH and NSF.)

**EFFECTS OF THE DOPA REACTION UPON DEVELOPING RETINAL AND EPIDERMAL MELANOCYTES IN THE FOWL.** J. A. BRENBAUGH AND R. H. ZIET (University of Nebraska, Lincoln, Nebr. 68508).

Ultrastructural observations confirmed the specificity of the dopa reaction. Retinal melanocytes from 3-day-old chick embryos (standard genotype), incubated in 5mM L-dopa for 3 hr. before fixation showed a significant increase in the melanosome/premelanosome ratio when compared with untreated tissue. Only premelanosomes within melanocytes reacted. Tissues fixed in glutaraldehyde (2 hr.) before treatment exhibited electron-opaque vesicles and Golgi cisternae as well as melanosomes. This "pre-fixed" reaction was very specific and demonstrated the role of the Golgi complex in melanin synthesis. The germs of regenerating feathers from both eumelanin and phaeomelanin producing regions of cockerels were also examined with the aid of the "pre-fixed" dopa reaction. Eumelanocytes were strongly dopa-positive. Their cytoplasm was crowded with large, electron-opaque, Golgi-related vesicles and melanosomes. Phaeomelanocytes were weakly dopa-positive. Their cytoplasm had only a few, small, electron-opaque, Golgi-related vesicles and premelanosomes. This reduced enzymatic capability may be responsible for the reduced melanin polymer found in phaeomelanin.

Developing retinal melanocytes of the pink-eyed (pk) fowl possessed dopa-positive Golgi complexes and premelanosomes. Thus the dopa reaction can be used to analyze gene action.


Three distinct, separable forms of active tyrosinase, T1 (EC 1.10.3.1) are present in extracts of mammalian melanoma: two (T1,T2) in the supernatant from an homogenate of tumor cells and a third (T3) bound to particulate material. T1 and T2 can be separated by electrophoresis; T3 can be solubilized with detergent. Preparations of T1 and T2 are judged to lack extraneous protein because there is a single, symmetric peak during determination of sedimentation velocity; the single elution profile from ion-exchange and/or molecular sieving chromatography is symmetric; and following acrylamide-gel electrophoresis the enzyme bands correspond to the protein bands under at least three different conditions of "running pH." T1 and T2 have very similar molecular weights, do not exhibit interconversion, and differ slightly, but significantly, in amino acid composition. This suggests that they are not simple polymers. Urea (8M) does not seem to effect the electrophoretic mobility or enzymic activity of either T1 or T2. When T1 and T2 are reduced and the excess reducing agent removed, enzymic activity is reduced or entirely lost; it can be partially restored or enhanced by oxidation. T1 and T2 retain their initial characteristic electrophoretic mobilities. By circular dichroism and optical rotatory dispersion, one can conclude that the active centers of T2 and T3 are probably independent of the molecular helix. This suggests that the active center of this ubiquitous enzyme may be small and stable. (Supported by USPHS Grants CA-09292 and CA-40110).

**DIFFERENTIATION OF PIGMENTED RETINA IN CLONAL CULTURE: CONTROL OF MORPHOLOGY AND GROWTH.** ROBERT D. CAHN, BRUCE CRAWFORD AND MARTHA B. CAHN (University of Washington, Seattle, Wash. 98105).

Dissociated single cells from the chicken pigmented retina can be grown in monolayer culture. Conditions of clonal culture
allow for the regular redifferentiation of a clone in a manner closely resembling certain developmental stages during normal eye development. Pigment production can be maintained even as growth occurs or reappeared at certain times depending on the culture conditions. During growth of the clone, several distinct areas can be defined. The central pigmented area eventually lifts from the petri dish. Light and electron microscopy indicate that this lifting is accomplished by secretion of a material resembling the vitreous somewhat in staining capacity. At the edge of the pigmented area, an area develops in which cells secrete large amounts of a somewhat different matrix material, containing both PAS-positive material and a strongly periodic macromolecular resembling collagen in the electron microscope. These epithelial cells synthesize large amounts of this collagen-like protein during certain stages of their growth and differentiation. The cells in this area pile up and form multiple cell layers. It is proposed from the data at hand that pigmented retina cells are responsible for the synthesis of vitreous material, and are the source of this material in vivo. (Supported by HD 05760-01, NIH; GB 7202, NSF; 65 G 102, AHA; and University of Washington GSRF Project 171 11-0061.)

ELECTRON MICROSCOPY OF MITOTIC HUMAN MELANOMA CELLS AND TISSUES. JEFFREY P. CHANG, TAKUMA SATO, MARVIN M. ROMSDAHL AND WILLIAM O. RUSSELL (University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025)

Fresh human melanomas from surgery were fixed in glutaraldehyde-osmium sequence and processed for electron microscopy according to standard methods. Some tumor materials were cultured, synchronized, flat-imbedded, or made into pellets for ultrastructural studies according to techniques developed in this institution.

It was found that the Golgi complex persisted in every stage of mitosis with the usual distinctive vesicular and laminar components, although their sizes and numbers had reduced. The fact that the Golgi appeared in more or less typical forms in metaphase is consistent with our previous observation on a transplantable hepatoma. (Chang and Gibley, Can. Res. 28: 531, 1968.) The view was macroscopically from some published investigations on other materials.

Formation of premelanosome was observed in both interphase and mitotic cells. Evidences suggested that these organelles were constructed from membranes which bent either inward or outward for usual three revolutions, into typical premelanosomes. In addition, the processes of formation of nuclear membrane and nuclear pores in telophase daughter nuclei were observed. Ultrastructural differences between the in vivo and in vitro materials will be presented. (Supported in part by USPHS Grant CA 11022.)

BASIC STUDIES OF THE PHYSIOLOGY OF THE MELANIN SYNTHESIZING PIGMENT CELL. WALTER CHAVIN (Wayne State University, Detroit, Mich. 48202)

The approach to the primary physiologic problems of the melanin synthesizing pigment cells of the goldfish is based upon the effects of the depigmentary agent, hydroquinone. Radiocarbon labeled hydroquinone was utilized to provide some cognizance of drug distribution pattern as a function of time after systemic administration. The major route of excretion was via the bile implying detoxification by the liver. The skin showed a relatively small uptake with rapid turnover. Using hydroquinone, black goldfish were depigmented, then injected with radiocarbon labeled L-tyrosine in order to pulse-label newly forming melanin in regenerating pigment cells. The cells were studied from 3 hr. to 330 days. Significant conversion to radiomelanin occurs within 3 hr. with the peak uptake of 12 hr. Total radiomelanin present in the skin at 310 days is approximately one-half of the 24-hr. interval. This suggests that the cells have a calculated life span of 310 days, but that a certain fraction of the population may be longer lived. At 630 days little or no radiomelanin is present in the skin. Skin and retinal melanin will be compared.

EFFECTS OF DEPIGMENTARY AGENTS AND RELATED COMPOUNDS UPON IN VITRO TYROSINASE ACTIVITY. YU-MIN CHEEY AND WALTER CHAVIN (Wayne State University and Michigan Cancer Foundation, Detroit, Mich. 48202)

A number of agents with unknown mode of action selectively destroy skin melanocytes and melanophores in the black goldfish and guinea pig. To evaluate drug effects at the enzyme level, the in vitro activities of purified mushroom tyrosinase, as well as tyrosinases in black goldfish integument, hybrid swordtail melanoma, B-16 melanoma, and human melanoma homogenates in the presence of depigmentational agents and related compounds were investigated. The 56 compounds tested revealed that the depigmentary activity of the drug is not always correlated with tyrosinase inhibition. Comparison of drug effects indicated both common characteristic responses and, perhaps, species differences in tyrosinases. Many reducing agents are potent tyrosinase inhibitors. Phenolic hydroxyl group position and existence of free sulfhydryl and/or amino groups are important. Both reducing and oxidizing agents may be inhibitors or activators depending upon drug concentration and/or enzyme source. The structural requirements for drug action upon tyrosinase are discussed.

CORRELATION OF MELANOSOMAL FINE STRUCTURE WITH MALIGNANCY IN HUMAN MELANOMAS. W. B. CLARK AND R. BRETTON (Temple University Medical Center, Philadelphia, Penn. 19140)

There are 3 gross and microscopic forms of human malignant melanoma, each different in evolution and behavior. This report will compare portions of 2 of these melanomas: tan-brown areas of lentigo maligna, tan-brown areas of superficially spreading melanoma, and deeply invasive areas of superficially spreading melanoma; these 3 melanoma samples have cells of different malignant potential, from essentially benign to potentially malignant to obviously malignant. Tan-brown lentigo-maligna cells show earlier melanosomal stages than normal; these melanosomes form unit filaments with periodicity and cross-striations. Tan-brown area of superficially spreading malignant melanoma show numerous melanosomes, most of which show unit filaments with periodicity but few cross-striations. Deeply invasive areas of superficially spreading malignant melanoma show organelles almost devoid of unit filaments, appearing as membrane delimited sacs with granular material having areas of electron opacity.

QUANTITATIVE STUDIES OF THE MELANOCYTES OF THE EPIDERMIS ADJACENT TO TUMOURS. ALISTAIR J. COCHRAN (University and Western Infirmary, Glasgow, W1, Scotland)

The incidence of basal layer melanocytes has been assessed in the epidermis overlying and adjacent to melanocytic and other tumors of and in the skin. The technique employed was direct counting of melanocytes in 6 μ vertical sections stained with hematoxylin and eosin. A raised incidence of melanocytes was observed over primary malignant melanomas, compound naevi, juvenile melanomas and lentigos. The normal area incidence of melanocytes was noted in the epidermis over simple intradermal nevi, blue nevi, secondary malignant melanomas, secondary carcinoma in the dermis and most simple and malignant non-melanocytic epidermal tumors. Three abnormal patterns of melanocyte distribution were noted around malignant melanomas: symmetrical field change, asymmetrical field change and no field change. Patients with tumors arising in areas of field change had a better 5-year survival rate and less frequent hematogenous dissemination than those with no evidence of field change.
THEORIES OF THE ROLE OF PIGMENT IN THE EVOLUTION OF HUMAN RACES. FARRINGTON DANIELS, Jr. (Cornell University Medical College, New York, N.Y. 10021) AND PETER W. POST (Columbia University, New York, N.Y. 10027)

*Homo sapiens* exhibits a wide variation in skin color. There have been many explanations. In theories pertaining to vitamin D synthesis, Europeans are light to promote vitamin D synthesis by ultraviolet radiation (UVR), while blacks are dark to protect from tropical UVR. Albinos in the tropics become sexually unattractive because of malignant and pre-malignant skin changes by puberty.

Jungle animals tend to be black, desert animals brown, and arctic animals white; protective coloration is usually implicit. The same has been hypothesized for man.

An inverse relationship between adrenal function and resistance to infection has been suggested; increased pigmentation in the tropics is considered mediated by lower adrenal function. An inverse relationship between adrenal function and resistance to infection has been suggested; increased pigmentation in the tropics is considered mediated by lower adrenal function. Fundus pigment and skin pigment correlate; light eyes have D synthesis, Europeans are light to promote vitamin D synthesis by ultraviolet radiation (UVR), while blacks are dark to protect from tropical UVR. Albinos in the tropics become sexually unattractive because of malignant and pre-malignant skin changes by puberty.

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The VITAL RESPIRATORY ROLE OF TYROSINASE IN PIGMENTED MELANOMAS. HARRY DEMOPOULOS, MARTHA A. G. REGAN AND DAVID REGAN (New York University Medical Center, New York, N.Y. 10016)

Previous studies have shown that known tyrosinase inhibitors can selectively depress respiration and growth of pigmented S-91 melanomas in *vitro* and *in vivo*. Amelanotic S-91 tumors which lack tyrosinase activity are not inhibited. Previous work suggested a coupling of tyrosinase to ox phos in the pigmented tumors, but this was indirect. In the present studies, direct measurements were made of the ATP levels of melanoma tissues incubated under the following situations: in S-91 melanotic and amelanotic tissues, with and without the tyrosinase inhibitors, phenyl lactate and p-OH phenyl lactate, at concentrations of 4, 6, 8 and 10 mM, at time intervals of 5 and 60 min. In the 60-min. incubations the O2 consumption was measured. The tissues were harvested and extracted at the end of each incubation and the ATP levels measured by the amount of TPNH generated after adding glucose, glucose 6-P04 dehydrogenase and TPN. There was a striking diminution in the ATP levels in the pigmented S-91 preparations that were incubated for 60 min, with the tyrosinase inhibitors. This diminution directly paralleled the degree of respiratory inhibition, both of which worsened as the concentration of tyrosinase inhibitors rose. The percentage inhibition of O2 consumption ranged from 27.9% to 48.9% while the percent diminution in ATP was 25.0% to 41.9%.

PROPERTIES OF TYROSINASE FROM THE PIGMENTED EPITHELIUM OF THE CHICK RETINA DURING DEVELOPMENT. PHILIP DOEZEMA (University of Washington, Seattle, Wash. 88165)

Measurements of tyrosinase activity per eye with respect to age of the developing embryo show that tyrosinase activity is first detectable at about 4 days of incubation, reaches a peak between 12 and 14 days and decays gradually to age of the developing embryo show that tyrosinase activity is first detectable at about 4 days of incubation, reaches a peak between 12 and 14 days and decays gradually. It is first detectable at about 4 days of incubation, reaches a peak between 12 and 14 days and decays gradually.

It has been shown that 0.5% sodium deoxycholate in 0.1 M tris buffer at pH 8.2 used as homogenization medium effectively releases tyrosinase into the soluble fraction with little initial loss of activity in crude homogenates.

When isolated melanin granules from 14-day chick retinas were treated with sodium deoxycholate a small amount of protein was released which contained nearly all of the tyrosinase activity. This protein and tyrosinase activity moved as a single band upon electrophoresis in acrylamide gels containing triton-x-100. (Supported by Grant GM 23192-02 from the USPHS.)

RELATIONSHIP OF A VIRUS TO MELANOMA TRANSPLANTATION IN GOLDEN HAMSTERS. WILLIAM L. EPSTEIN, KIMIE FUKUYAMA AND JIMMIE HIGASHI (University of California School of Medicine, San Francisco, Calif. 94122)

We have maintained a transmittable melanoma by subcutaneous injection of a cell-free supernatant of a spontaneous hamster melanoma. This report concerns an attempt to correlate the early proliferative events of the melanoma with the primary cell-free supernatant of a spontaneous hamster melanoma. This report concerns an attempt to correlate the early proliferative events of the melanoma with the primary cell-free supernatant of a spontaneous hamster melanoma. The inocula are prepared by hand homogenization of the melanoma followed by centrifugation at 300 or 800 g for 8 min. The supernatant, sedimented at 10,000 g, reveals no intact cells, but melanosomes (m), premelanosomes (pm), various membranous structures, and VLP are seen. Most of the cellular particles degenerate within 5 days after injection and the area is surrounded by phagocytes filled with melanin granules. By 7 to 10 days the tissue is vascularized and fibroblasts, mononuclear cells, mast cells and occasional giant cells appear. Small basophilic mononuclear cells, rich in endoplasmic reticulum, also are seen, sometimes in small groups, but no VLP or premelanin granules are observed in these cells. At 14 days nests of malignant cells containing VLP and premelanosomes but no melanosomes are detected. Cells in division contain VLP, indicating a mode of transmission. The early appearance of VLP correlates with the onset of malignancy and precedes melanin production.

IRIDOPHORES IN THE IRIS OF DOVES, WAYNE R. FERRIS AND JOSEPH T. BAGNARA (University of Arizona, Tucson, Ariz. 85721)

The Mexican Ground Dove and the Inca Dove display striking eye color changes which are in part based upon the presence of iridophores in the iris. Since this type of pigment cell has never been described for warm-blooded animals it was necessary to identify these cells using parameters established for the identification of these cells in lower vertebrates. Iridophores of the dove iris comprise a dense reflecting and birefringent mass in the central and posterior stroma. Electron microscopic observations reveal that these cells are filled with reflecting platelets arranged at random. Extracts of the iris are rich in free purines (especially quanine) and following cytolyis of the iris crystalline structure like those of amphibian iridophores can be recovered. Another type of reflecting pigment cell occurs in the anterior portion of the iris in association with capillaries. Its cytoplasm is filled with densely packed birefringent bundles of parallel rod-shaped (4-10 μm × 60-100 μm) reflecting elements. The colors they reflect suggest that this element serves as a diffracting element.

PREPARATION OF FRACTIONS OF PREMELANOSOMES AND MELANOSOMES FROM PIGMENTED S-91 MELANOMAS. HENRY FIERMAN AND HARRY DEMOPOULOS (New York University Medical Center, New York, N.Y. 10016)

Efforts to isolate premelanosomes and melanosomes from Cloudman S-91 melanomas have previously met with unfruitful results. Others have tried to eliminate mitochondrial contamination of melanosome fractions by increasing the density of melanosomes by "extra-melanization." In this work sucrose homogenates of well pigmented S-91 tumors, cleared of unbroken cells and nuclei at 2000 × g were layered directly onto sucrose density gradients, 1.0-3.0 M (without preparing a large granule fraction) and centrifuged at 100,000 × g for 75 min. at 5°C in a Spinco Model L. Fractions were drawn off and prepared for electron microscopy. Three melanosome-rich fractions, reflecting different degrees of melaninization and free of mitochondria, were obtained. The fine structure of premelanosomes and melanosomes was well preserved. Omission of the step preparing a pellet of large granules is felt by the authors to have minimized adherence of particles and
to have fostered the separation obtained. Other factors that were probably important in the present separation were a lower initial dilution and elimination of washing and resuspension procedures to avoid a dialysis of organelles and alterations of the surface charges of the melanosomes and mitochondrion.

STUDIES OF RED PIGMENTARY SYSTEMS. Peter Flesch (Univ. of Pennsylvania, Philadelphia, Penn. 19104)

Red pigments ("trichosiderins") were isolated from man, dog, rabbit, hamster, rat, mouse and chicken. Iron is an essential part of these pigments because when prepared under the mildest conditions ("protopigments") or with boiling acids ("siderins"), these substances are split by iron-combining anions. After such splitting the chromophore-peptides become dialyzable and their iron content rises to 1.5%. As the chromophore is destroyed by Fe\textsuperscript{III}, a secondary combination or contamination with Fe\textsuperscript{III} is unlikely. Because of the common precursor dopa, pigment formation may be diverted into red or black pathways by regulating catechol or dopa uptake. We produced a local red-black switch by vigorously rubbing New Zealand red rabbits. The presence of siderins may presuppose the overt or latent ability to form black pigments and to effect their turnover in aged animals (hamster, black, rat, mouse). Here the switch is physiologic; in the black phase no red pigment is produced. A black-red switch may cause the red discoloration of protein-deficient children in Biafra. These findings have evolutionary implications.

GENETIC CONTROLS OF MAMMALIAN MELANOGENESIS. Morris Foster, Elizabeth Barto and Lucinda Thomson (University of Michigan, Ann Arbor, Mich. 48104)

A number of color mutants in the house mouse (Mus musculus) and deer mouse (Peromyscus maniculatus) have been tested for melanogenic lesions. In both species the genes for albinism (c), brown (b), dilute (d) and pink-eye (p) seem to regulate melanogenesis in similar ways. The ivory (i) locus in Peromyscus may have no homologous counterpart in Mus.

These and other observations can be explained as follows: The albinism (c) locus serves as the structural gene for tyrosinase which, in concert with other color gene-specified matrix proteins, participates in melanosome construction and function. Altered melanogenetic attributes due to mutation at loci other than c can be viewed as secondary effects involving abnormal tyrosinase conformation resulting from bonding of tyrosinase with normal primary structure to various abnormal matrix proteins. Such mutations can also lead to altered melanosomal architecture and melanin deposition. (Supported in part by Grants HD-01259 and NB-00905 from the NIH of the USPHS and by GB-7241 from the NSF.)

THE NERVOUS CONTROL OF MELANOSOME MOVEMENTS IN Vertebrate MELANOPHORES. Ryoko Fujii (National Institute of Radiological Sciences, Chiba City, Japan) and Ronald R. Novalis (Northwestern University, Evanston, Ill. 60201)

Nervous control of melanosome movements in melanophores is found in teleost fishes and some reptiles. The most precisely studied system is the melanin-aggregating nervous supply in teleosts. Electrical stimulation of nerves causes the liberation of chemical transmitter from presynaptic elements of the melanin-aggregating nerve fibers. Alkaline-earth ions are required in the urine for release of the transmitter. The transmitter may be of the adrenergic type, because adrenergic blocking agents effectively block transmitter action.

Even a single electrical shock to the nerves can induce moderate melanin aggregation. This response is not of the all-or-none type and its magnitude increases upon increase in stimulus intensity. Thus, each melanophore may receive several such fibers. Furthermore, recent ultrastructural studies show that there may be 2 kinds of fibers, possibly the melanin-aggregating and dispersing types. The presence of a dispersing innervation is also suggested by some physiological investigations, although the chemical nature of the transmitter is still unknown. (Supported by NSF Grant G-4956X.)

ACID MUCOPOLYSACCHARIDES CONTAINING NEURAMINIC ACID IN THE CYTOPLASM OF THE MELANOPHORES OF BUFO SPINULOSUS WIEGMANN. Humberto Garcia, Ximenia Fischer, Fritz Lébel and Juan Vergara (School of Medicine, University of Chile, Santiago, Chile)

It has been suggested that the rapid movements to dispersion and aggregation of melanin granules in melanophores are due to gelation and solution in the cytoplasm. The hypothesis leading to the present paper is that this phenomenon could be due to polymerization and depolymerization of mucopolysaccharides. In sections of skin of the frog, Bufo spinulosus Wiegmann, it was possible to demonstrate that the cytoplasm of the melanophores and lipophores contains a mucopolysaccharide rich in neuraminic acid. A study of the ultrastructure of the cytoplasm of the pigmentophores revealed the presence in the melanophores and lipophores of fibrillar aggregate of 200 Å of variable length, and also granular elements of similar dimensions. Both types of elements probably correspond to the mucopolysaccharides demonstrated histochemically.

HORMONAL MODIFICATION OF COAT COLOR IN THE LABORATORY MOUSE. Irving L. Geschwind (University of California, Davis, Calif. 95616) and Robert A. Huseby (American Medical Center at Denver, Spivak, Colo. 80214)

Previous experiments with mice of differing genotypes have demonstrated that MSH darkens coat color solely by affecting the expression of the agouti locus, producing an effect which is best seen in the yellow mouse (A\textsuperscript{q}). The color of the recessive yellow (ee) is not affected by MSH, and recent experiments reveal that mice of the genotype A\textsuperscript{q}a ee are also unresponsive. The effect can be detected within 12 hours in actively growing hair as an appearance of eumelanin granules in the follicles, and preliminary findings indicate that tyrosinase activity in skin is markedly increased in that time (assays performed by Dr. Walter Chavín). Other experiments have been conducted to determine the effects of colchicine, cycloheximide, and actinomycin D on eumelanin synthesis in MSH-treated A\textsuperscript{q} mice. The first 2 prevent darkening, whereas actinomycin D apparently does not. We will report the results of further experiments with these agents and with puromycin, and discuss the effects of MSH administration on the activity and multiple forms of tyrosinase in skin, and on the electron microscopic appearance of premelanosomes in the skins of 5-day-old mice.

EPIDERMAL MELANOCYTE PROLIFERATION. Luigi Giacometti (Oregon Regional Primate Research Center, Beaverton, Ore. 97005)

Epidermal melanocytes are capable of proliferating in skin wound healing and after U.V.-irradiation. In small wounds made on the scalp of rhesus monkeys (Macaca mulatta), from 2 to 3 days after wounding, we found melanocytes in the vicinity of the cut and behind the advancing epithelial sheets. The onset of the melanocytic proliferation coincided with the epithelial closure of the incisional gap at day 6; from days 6 to 9 after wounding, melanocytes were found in all stages of mitosis. Melanocytic proliferation has also been confirmed in skin wound healing in guinea pigs. Using tritiated thymidine and autoradiography, we found that 6 days after wounding when the incisional gap had been bridged by the epithelial tongues, tritiated thymidine was incorporated in some epidermal melanocytes at the edges of the wounds. Thus, in skin wound healing melanocytes migrate and proliferate only
when the epidermis continuity is re-established and their movement is directed into preformed sheets of epidermis. That epidermal melanocytes are capable of proliferating after exposure to U.V. light has also been found. Preliminary studies in our laboratory have indicated that after repeated U.V. light irradiation, epidermal melanocytes in the rhesus monkey (1) synthesize increased amounts of melanin pigments; (2) proliferate in response to U.V.

INNERVATION OF THE FROG'S PARS INTERMEDIA AS A REGULATOR OF MSH RELEASE, Aubrey Gordon, Yasumitsu Nakai and Kiyoshi Oshima (University of Washington, Seattle, Wash. 98165)

In recent experiments it was found that 2 different classes of spontaneously electrically active neurones innervate the frog (Rana pipiens) pars intermedia, one light-inhibitable and the other light-indifferent. Using electron microscopy, we found further that 2 and possibly 3 kinds of nerve endings terminate synaptically on secretory cells of the pars intermedia. One cell may bear either 1 adrenergic endings (characterized by dense-cored vesicles), or one adrenergic and one "neurosecretory" (large dense vesicles) ending. In further experiments lesioning procedures followed by study of degenerative processes in endings in the pars intermedia show that the neurosecretory and adrenergic fibers come from separate sources beginning near the preoptic nucleus. An interesting phagocytic behavior of MSH-secretory cells toward degenerating nerve endings was found. These data have been incorporated into a hypothesis of double opposed secretomotor nervous control over MSH-secretion and/or release in the frog.

MELANOGENESIS IN THE DECAPOD CRUSTACEAN UCA PUGNAX, Jonathan P. Green (Brown University, Providence, R.I. 02912)

Epidermal activity in the fiddler crab involves pigment cells in which two distinct phases of behavior are evident: (1) a rapid mobilization of the pigment leading to its migration within pre-established cellular channels, (2) a slower modification of the quantity of the pigment. In part, the present work reports the fine structure of the crustacean epidermis with emphasis on the melanophore.

Autoradiographic studies of the in vivo incorporation of [H]tyrosine into melanin indicate that only the melanophore is involved in pigment synthesis. With in vivo techniques, only major differences in melanin synthesis are detectable. The use of an in vitro membrane assay (Chen and Chavin 1965) allows the detection of more subtle variations in melanin synthesis. Basic parameters of enzyme activity such as optimum pH, temperature, etc. have been determined. Significant differences in activity exist between the two locally-occurring species of crabs. The activity of tyrosinase preparations from a random selection of crabs appears to fall in distinct classes. This variation reflects general changes in epidermal metabolism prior to ecdysis. The present work substantiates the hypothesis that melanogenesis in crustacea is correlated with the moulting cycle.

THE PHYSIOLOGICAL REGULATION OF THE AMPHIBIAN IRIDOPHORE. Mac E. Hadley and Joel M. Goldman (University of Arizona, Tuscon, Ariz. 85721)

Iridophores, like melanophores, play an important role in integumental color changes of amphibians. These reflecting pigment cells are regulated by the same hormones that control melanophore function. A study of these cells provides a further insight into the physiology of melanophores. Iridophores are normally regulated by intermedin which causes a perinuclear aggregation of reflecting platelets within these cells. The action of intermedin on iridophores can be reversed by: acetylcholine (acting on a cholinergic receptor), catecholamines (through an alpha adrenergic receptor), and triiodothyronine. Melatonin, in contrast, reverses the action of intermedin on melanophores but not its effect on iridophores. MethyIxanthines (caffeine and theophylline) and cyclic AMP and its dibutyryl derivative have intermedin-like effects on iridophores suggesting a possible role for cyclic AMP in iridophore regulation. Comparative studies of iridophore (and melanophore) responses to hormonal stimulation reveals interesting inter-species as well as intra-species variation which in some cases (catecholamine stimulation) is related to the presence or absence of alpha adrenergic receptors possessed by the chromatophores.

THE INFLUENCE OF OESTRADIOL ON MELANOGENESIS IN FEATHERS OF THE BROWN LEGHORN. Peter F. Hall (University of Melbourne, Victoria, Australia)

A recent report indicated that red feathers contain an acid-soluble pigment which contains cysteine. Brown leghorn chickens show a sex dimorphism with respect to plumage color, the result of which neck feathers in the male are black, and those of the female salmon pink; the pink color results from the action of estrogens. When estradiol is injected daily for 3 days (10 microgram/chicken), tyrosinase activity is observed 24 hours after the last injection (60 microgram tyrosine hydroxylated per mg. protein per hour). This response is prevented by injection of puromycin (20 microgram/g body weight) if injection is commenced 24 hours before the first injection of estradiol. These results are compatible with the hypothesis that the response to estradiol requires synthesis of some protein involved in melanogenesis. When melanosomes fractions from feather tracts of leghorn chickens were incubated with tyrosine-3H or cysteine-35S, an acid-soluble pigment was isolated from female birds or male birds treated with estradiol but not from untreated males. Moreover, cysteine-35S and tyrosine-3H, but no other amino acids, were incorporated by melanosomes into this pigment.

THE DEVELOPMENTAL GENETICS OF TYROSINASE WITHIN THE MELANOCYTES OF MICE. T. J. Holstein, J. B. Burnett and W. C. Quevedo, Jr. (Brown University, Providence, R.I. 02912 and Harvard Medical School, Boston, Mass. 02114)

The regulation of melanin synthesis may be intimately associated with variations in the types of tyrosinase produced by mouse melanocytes. It is now clear that tyrosinase exists within follicular melanocytes in multiple forms which are separable by acrylamide gel electrophoresis of crude extracts of pigmented hair bulbs. Extensive allelic substitutions at the a, b, c, d, and p "color" loci in mice reveal that the multiple forms of tyrosinase are subject to complex genetic control. Depending upon genic constitution, a maximum of 3 electrophoretically separable forms of tyrosinase (Ti = 80,000 m.w., Ts = 61,000 m.w., and Ta = 63,000 m.w.) are demonstrable. In certain genotypes of mice, Ti, Ta, and Ts are either greatly reduced in activity or absent. The appearance and relative intensity of the multiple forms of tyrosinase within dopa-treated acrylamide gels are dependent upon the phase of the hair growth cycle at which hair bulbs are obtained. The electrophoretic patterns of tyrosinase obtained from eyes of pigmented mice differ from those found for hair follicles. Collectively, the results suggest an adaptive function. At present, however, it is not certain whether the multiple forms of tyrosinase are true isoenzymes or rather a single enzyme moiety bonded to carrier proteins. (Supported in part by USPHS, NCI Grants CA-08292 and CA-06097, and training grants GM-00832 and HD-00019.)

RADIOGRAPHIC ELECTRONMICROSCOPIC STUDY OF CHICK EMBRYONAL RETINAL PIGMENTED EPITHELIUM. Yosihaki Hori (University of Tokyo, Tokyo, Japan)

Retinal pigment epithelium of 8-day-old chick embryos is involved in pigment synthesis. When melanosomes from feather tracts of leghorn chickens were incubated with tyrosine-3H or cysteine-35S, an acid-soluble pigment was isolated from female birds or male birds treated with estradiol but not from untreated males. Moreover, cysteine-35S and tyrosine-3H, but no other amino acids, were incorporated by melanosomes into this pigment.

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el ect ron microscopy. The distribution of silver grains in radio­
were found on ribosomes, rough and smooth membranes, and melanosomes in all stages of melanization. At 3 hr., grains were found on rough and smooth membranes, and on melano­somes in all stages of melanization. At 6 hr., grains were mostly on melanosomes in late stages of melanization. Most of the grains were localized on melanosomes in exposure to dopa-H 3 , almost all grains were on fully melanized melanization; at 24 hr. , mostly on fully melanized melano­some. The incorporation of dopa-H3 by ribosomes suggests
that the synthesis of tyrosinase may be transferred through the endoplasmic reticulum of Golgi complex and to melano­some where melanization finally occurs.

FACTORs INFLUENCING MELANIN PRODUCTION
IN CELL CULTURE. F. U. HU (Oregon Regional Primate Research Center, Beaverton, Ore. 97005)
A study of the growth behavior of the pigment cell strain HFF-18 in culture suggests that conditions favoring cell proliferation tend to inhibit melanin production and those re­tarding growth encourage pigment formation. Frequent sub­culturing and feeding decrease the number of pigment-con­taining cells. In these cells, pigment production is favored by prolonged incubation with occasional feeding, inhibition of mitosis by growing cells at cold temperature and in the presence of mitotic inhibitors, and growing cells in aggregates or under a dialysis membrane. Co-cultivation with cells of dif­ferent species also alters the number of pigmented cells. How these conditions affect melanin pigmentation will be discussed.

TRANSPLANTABLE MELANOTIC TUMORS INDUCED IN AxC RATS BY A SINGLE FEEDING OF DMB A.
RIBBERTO I GLE SIAS AND SOCORRO SALINAS (Instituto de Medicina Experimental del Servicio Nacional de Salud, San­tigo, Chile)
Mammary cancer has been induced in 100% of intact, 60­day-old female Sprague-Dawley rats, following a single feed­ing of 20 mg. of 7,12-dimethylbenz(a)anthracene (DMBA) (Hughes et al., Nature 189: 294, 1961). We repeated these ex­periments, using AxC (brown) rats. Of 40 intact female AxC rats treated, 31 survived from 259 to 569 days. Mammary tumors developed in 20 of these animals, 150 days being the shortest latent period. However, in all 31 animals, pigmented tumors of the skin appeared, some of them pitch-black, others, bluish-black and others, grey. The latent period was from about 175 to 400 days. The size of the tumors was from 1 to 13 mm.; the tumors were located especially on the head, but there were tumors in various locations, including the tail. Tumors from 3 of these animals were transplanted into fresh AxC rats. One did not grow, another grew in one gen­eration, and the third was lost in the fourth transplant generation. Among 35,469 AxC rats autopsied, only 11 melano­nomas have been found.

COMPLEMENT DEPENDENT INHIBITION OF RNA SYNTHESIS IN MELANOMA CELLS. RUPUS L. IKONOPIS­sov (Oncological Institute, Sofia, Bulgaria)
Cytotoxic antibodies in the serum of patients with malig­nant melanoma were assessed. The technique employed is based on the principle that RNA synthesis of melanoma cells in suspension can be estimated by the uptake of [3H]uridine after incubation with autologous, homologous or normal control sera, with and without added complement. The number of

semitations resulting from each specimen was inversely proportional to the cytotoxic effect of the sera upon the melanoma cells.

The sera of 44 melanoma patients were tested against their own tumors. Autologous cytotoxic effects were observed in approximately 30% of the cases. Cross reactions were seen in a limited number of cases. Autologous cytotoxicity diminished with the spread of the tumor, and at times increased with the removal of the tumor mass or following immunological (specific and nonspecific) procedures. The [3H] uptake cyto­toxicity test may therefore provide a diagnostic and prog­nostic tool for therapeutic planning in the approach to human malignant melanoma, based upon the immunological status of the host-tumor relationship.

MELANIN AND CELL REACTIONS TO ULTRAVIOLET RADIATION (UVR). B. E. JOHNSON, G. MANDELL AND F. DANIELS, Jr. (Cornell University Medical College, New York, N.Y. 10021)
Melanin in human epidermis affords protection against UVR damage to underlying cell and tissue elements. However, in many "sunburn cells" typically found in Caucasian epidermis 12-48 hours after irradiation, we see more melanin than in neighboring, apparently undamaged cells. In viti­go, we have produced fewer "sunburn cells" at all dose levels in a dose response study than in normal Caucasian skin. Further, we find that macrophages containing squid ink melanin are more susceptible to UVR killing than control macrophages. In Caucasian keratinocytes, melanosomes are associated for at least some time with lysosomes. The association appears similar to that of bacterial and leukocytic lysosomes. We propose that when melanosomes enter keratinocytes, secondary lysosomes are formed with characteristically increased membrane fragility. The more melanin incorporated, the more fragile the membranes and the greater the susceptibility to rupture by UVR, with subsequent enzyme release and auto­lysis. In the special case of the "sunburn cell," melanin may therefore supplement rather than decrease the damaging effects of UVR.

In gel filtration experiments, mushroom tyrosinase was ob­served to exist in the tetramer, dimer, and monomer forms, often simultaneously present in the same preparation. Over the range of experimental conditions employed, the associa­tion-dissociation equilibrium among the 3 forms was found to be slow. No dilution-induced dissociation was observed, even at high dilutions. From this work it is concluded that the tetramer, at least, is an active species toward tert-butyl­catechol.

Band sedimentation of tyrosinase in a medium containing tert-butylicatcatala revealed an active species with an s20,w of around 2.5S, corresponding to the monomer. Bands corre­spoding to associated forms were not observed.

The prosthetic copper of mushroom tyrosinase did not bind CO in the absence of substrate (catechol), but in the presence of catechol CO was bound in the ratio of about 1.0 CO per atom of copper.

The data thus far obtained indicate that all polymeric species are enzymically active as such.

MSH RELEASE IN MAMMALS. ARB J. KASTIN AND ANDREW V. SCHALLY (VA Hospital and Tulane University School of Medicine, New Orleans, La. 70140)
Evidence has been accumulating during the past 5 years which indicates that the predominate control of melanocyte-stimulating hormone (MSH) release in mammals is exerted by an inhibiting factor (MIF) of hypothalamic origin. Thus,
Progress in purification and separation of 2 pigmentary-effector hormones from the crustacean eyestalk is reported. One hormone causes light-adaptation of the distal retinal pigment (DRPH) and the other (MDH) disperses melanin pigment in brachyuran melanophores. Both DRPH and MDH occur in the same chromatographic zone after filtration on columns of G-25 Sephadex. Successive chromatography of this active material on columns of anion (DEAE cellulose) and cation (CM cellulose) exchangers reveals 4 apparently different molecular forms of MDH which may be isohormones. DRPH activity consistently appears in the same chromatographic fractions containing MDH (3 tested). Such consistent identity of chromatographic behavior by DRPH and MDH indicates that they may be closely related molecules, or even that the 2 pigmentary effectors are activated by the same substance occurring in several different molecular versions.

DEVELOPMENTAL PHYSIOLOGY OF EARLY PIGMENT CELLS IN THE AVIAN EMBRYO AND THE TRANSFER OF MELANIN-PIGMENT INTO FEATHER CELLS. H. U. KROECKER (Zoological Institut of University, Kiel, Germany)

Melanoblasts of black Khaki Campbell duck embryos start to synthesize pigment at 4.5 days of incubation, when they reach the mesenchyme above the midbrain or ear vesicles. These cells can be differentiated from mesodermal cell by their high content of RNA, using the gallocyanin-Chrome alum technique. Melanoblasts possess numerous polysomes, and the endoplasmic reticulum is in connection with the nuclear envelope. Neural crest cells can be identified at the margin of the neural plate as early as the 2-somite stages. The early melanoblasts do not form typical premelanosomes, but start the synthesis in irregular clusters of vesicles. Later the synthesis takes on a regular pattern. The cells of feather germs engulf small parts of melanoblastic pseudopodia, after which the membranes of the feather cell and of the engulfed pseudopodium fuse.

INHIBITION OF THE GROWTH OF MURINE AMELANOTIC MALIGNANT MELANOMA WITH POLYINOSINIC-POLYCYTIDYLIC ACID. A. W. KOPP AND R. S. BARTT (New York University School of Medicine, New York, N.Y., 10016)

Synthetic double-stranded ribonucleic acid (poly I. poly C) in diluent was injected intraperitoneally into C-57 mice bearing recently transplanted B-16 malignant melanomas. The amount administered was 150 µg. per dose for 7 to 9 injections. Control mice with the tumors received either no injections or the diluent alone. About 2 weeks after transplantation, the mice (then about 8 weeks old) were killed and the volumes of their tumors measured. The experiment was performed 3 times with a total of 60 animals. In the 3 groups of mice treated with poly I. poly C the average volumes of the tumors were 1.7, 1.1 and 0.6 ml. The corresponding average volume in the control groups were 2.9, 2.2 and 1.7 ml. respectively. The inhibition of growth by poly I. poly C was statistically highly significant (P < 0.005). The effect was shown not to be due to inhibition of tyrosinase.

NERVOUS CONTROL OF CHROMATOPHORES IN CEPHALOPODS. MARION E. KRIEBEL AND ERNST FLOREY (Upstate Medical Center, State University of New York, Syracuse, N.Y., 13210 and Universitaet Konstanz, Germany)

Cephalopods are capable of extraordinarily rapid color changes. Each of their red, yellow and brown pigment cells has its own musculature: a set of radially arranged muscle fibers that insert at the equator of each lens-shaped chromatophore. The color pattern of the skin is under the control of the central nervous system since the muscle fibers receive a motor innervation. In addition, the muscle fibers can undergo spontaneous contractions. Microelectrode studies re-
revealed that these are caused by spontaneous quantal release of transmitter substance from motor nerve terminals. This release is enhanced by externally applied acetylcholine and decreased by 5-hydroxytryptamine. While the muscle fibers show no electrical signs of a direct action of these substances on their cell membrane, the nerve terminals show the pharmacological responses of a typical cholinceptive structure. No evidence was found to suggest that ACh is the transmitter substance of the motor neurons.

The spontaneous pulsations of the chromatophores in the skin of squid (Loligo) are caused by muscle twitches due to spike potentials arising spontaneously in the muscle fibers of the motor neurons. Nearby muscle fibers are electrically coupled so that spikes can be conducted throughout the musculature of the chromatophore. Motor nerve stimulation produces only local postsynaptic potentials. (Supported by NIH Grant NDB 1451.)

ATTENUATION OF MALIGNANT MELANOMA ASSOCIATED WITH LOSS OF PREMELANOSOMES (PMS) FOLLOWING POX VIRUS ONCOLYSIS—A REPORT OF TWO CASES. MALCOLM LANE-BROWN (University of Sydney, N.S.W., Australia)

Previous studies have shown that massive doses of pox viruses injected into melanomatous tumors could attenuate the power to synthesize premelanosomes prior to treatment. This loss of premelanosomes was accompanied by diminution of the aggressiveness of the tumors. Although there were no demonstrable PMS in the tumors, dopa and tyrosinase-positivity remained, and tissue slices incubated with dopa produced a melanin-like substance. Atomic absorption spectrophotometry showed that the pure substance contained zinc and copper, thus indicating that it could not have been formed by the autoxidation of dopa.

SUNLIGHT AND MALIGNANT MELANOMA IN HUMANS. JOHN A. H. LEE (University of Washington, Seattle, Wash. 98105)

The correlation of an increased death rate from malignant melanoma in white humans with decreasing latitudes of their residences has been observed. This suggests that exposure to sunlight is an important etiological factor in melanoma of Caucasians. These differences between latitudes are observed in young adults, but do not increase with age, implying that this effect of sunlight is not cumulative. However, there is no relationship between the high incidence of typical invasive melanoma in the tropics and exposure of the site of the primary tumor to sunlight. In contrast, superficial lentigo maligna occurs preferentially on exposed parts of the body, and in the middle-aged and elderly, implying a direct cumulative effect of sunlight. Sunlight is therefore postulated to play two separate roles in the etiology of malignant melanoma in Caucasians.

INDUCTION OF PIGMENT IN HAMSTER AMELANOTIC MALIGNANT MELANOCYTES BY RNA FROM A MELANOTIC STRAIN. GEORGE LIPKIN (New York University School of Medicine, New York, N.Y. 10016)

A report of in vitro pigment formation of hamster amelanotic malignant melanocytes by DNA from melanotic tumor prompted studies of effects of "melanotic" RNA in a similar system. It was shown that temporary non-heritable pigment induction could be achieved in a small proportion of amelanotic cells incubated for 30 minutes at 4°C, 20°C, or 37°C, under isotonic or hypotonic conditions in the presence of 40-600 μg/ml of "melanotic" RNA. Pigment granules first appeared at 48 hours, fully pigmented cells by 9 days, and the maximum population of pigmented cells by 18-21 days. Thereafter, as cells divided, pigment diminished and by 8 weeks most had disappeared. Pretreatment of "amelanotic" RNA with RNAase prevented pigment induction. Addition to incubations of Dextran DEAE (100 mg/ml) or Neomycin (.25 mg/ml) augmented pigmentation. Multiple defects in melanogenesis have been reported in hamster amelanotic malignant melanoma; "melanotic" RNA may temporarily correct these. Pigment induction may provide a useful experimental tool for probing the molecular bases of various malignant and benign pigmentary disturbances.

ULTRASTRUCTURE OF GIANT MELANIN GRANULES IN THE BEIGE MOUSE DURING ONTOGENY. MARVIN LOWRY (National Cancer Institute, NIH, Bethesda, Md. 20014)

The beige mouse, homozygous for the (bg) gene appears to be the murine counterpart for the human with Chediak-Higashi syndrome (CHS), the CHS (Aleutian) mink, and the CHS cow. CHS is characterized by autosomal recessive inheritance, dilution of eyes, hair and skin pigment, giant cytoplasmic granules, especially those of the melanocyte and leukocytes, and susceptibility to infection. In order to study the fine structural development of this gene-induced granule malformation, eyes of embryonic and adult beige mice were prepared for electron microscopy. The giant cytoplasmic melanocytes of the retina contained a melanin-like pigment that were observed. CS7 black (wild type) mice were studied as controls. The earliest observable melanin granules in the retina and choroid of beige embryos were larger than control granules. Granule membranes were disrupted and melanosome fibers were surrounded by vacuolar spaces. In older beige embryos giant granules were seen with a central nidus of normally formed melanin cylinders surrounded by layers of melanin fibers, exhibiting a spectrum of maturity with the least mature fibers towards the periphery. Premelanosomes appeared to be fusing to the outside of these giant granules. In adult melanocytes the cytoplasm appeared filled with mature giant granules. These studies suggest that the beige gene defect might involve malfunction of size control mechanism, through the continuous fusion of premelanosomes to more mature granules.

REGIONAL PATTERNS IN MORBITDITY FROM MELANOMA IN TEXAS, 1944-1966. ELEANOR J. MACDONALD, PATRICIA F. WOLF AND MARY S. JOHNSON (M.D. Anderson Hospital and Tumor Institute, Houston, Tex. 77030)

In a 74-county in-depth study in Texas of the age-adjusted incidence rates for melanoma based on their proportional number among 250,000 accessions from total cancer during a 23-year period, 1944-1966, strong regional patterns were found, both in level of morbidity rates and in their variation over time. Marked differences were found between males and females, between Latin surnamed Caucasians and other Caucasians, as well as between Caucasians and Negroes, not only in the changing incidence rates for each site, but in the age pattern of these rates. Linear and logarithmic trends were fitted for the full 23-year period. In addition to regression analysis over time, correlation analysis was applied to the data from hospitals in each of the 6 large natural hospital regions in different parts of the state, into which the 74 counties combine, and for each site of cancer. Relationships between various sites of cancer and melanoma in the regional pattern were derived, as well as similarities with respect to the changes that occurred in their morbidity rates over this period. The method of obtaining these data included examination of every record in dermatologists' offices with the indexing and abstracting of every cancer case as well as in hospitals, clinics, laboratories, and group practices, which resulted in practically total inclusion of all melanomas diagnosed in this definable population of 4 million with 3 ethnic groups upon which the study is based.
THE “ACTIVE” JUNCTION NEVUS. DEAN MARTALOCK AND R. K. WINKELMANN (Mayo Clinic, Rochester, Minn. 55901)
The diagnosis of “active” junction was studied in a group of 45 patients of the Mayo Clinic in whom this diagnosis was made histologically from 1950 to 1963. Restudy permitted classification of the lesions into an “active” junction group of 36 cases and a suspect melanoma group of 9 cases. All but 1 case was followed 5 or more years. The diagnosis of “active” junction nevus had been used if signs of epithelial hyperplasia and hyperpigmentation, clear, large cells with prominent nuclei in the epidermis and spindle cell junction activity, giant nevus cells or mitoses were present. One of 35 “active” junction nevus cases resulted in melanoma. Five of the 9 suspect melanoma cases resulted in metastatic melanoma disease. Histologic identification of changes of “active” junction nevus indicates that complete histologic study of the lesion must be made. Local excision is sufficient treatment if the diagnosis is verified.

THE PTERINOSOMES: A SUBCELLULAR UNIT IN THE BRIGHTLY COLORED PIGMENTATION OF LOWER VERTEBRATES. JIRO MATSUMOTO (Keio University, Yokohama-Hiyoshi, Japan, and the University of Arizona, Tucson, Ariz. 85721)
Yellow or red colored pigment cells, xanthophores and erythrophores, play a fundamental role in brightly colored integumental pigmentation of lower vertebrates. The principal pigments in these cells are either pteridine or carotenoid or both. The ultrastructure of the differentiated xanthophores is characterized by the presence of pigment granules and smooth endoplasmic reticulum. Combined biochemical and electron microscopic studies show that pteridines are characteristically located in a specific cytoplasmic organelle termed the pterinosome and that carotenoids are found in the endoplasmic reticulum or in cytoplasmic vesicles. Pterinosomes are defined by a limiting membrane and contain an internal structure characterized by either concentric lamellae or an irregular system of fibrils. The former, rich in drosopterins, are found in Xiphophorus fishes and in a variety of amphibian species, whereas the latter, laden with sepiapterin and other porphyrins, are seen in goldfish or in color mutant of a frog. The activity of tyrosinase, which probably exists in a masked state, is recognized in the both types of pterinosomes.

MITOSIS IN HUMAN MELANOFA C CELLS IN VITRO. GERR G. MAUL (Temple Medical Center, Philadelphia, Penn. 19140)
This is a study of very active melanotic melanocytes grown in tissue culture. Mitotic cells were selected under the light microscope and prepared in situ for electron microscopy. Since it had previously been found that premelanosomes are connected to the Golgi apparatus through the smooth surfaced ER, at least for part of their genesis, special attention was paid to the breakdown and reformation of the Golgi apparatus. The hypertrophied Golgi apparatus starts to was paid to the breakdown and reformation of the Golgi apparatus. The hypertrophied Golgi apparatus starts to

CONTROL OF MELANOBlast DIFFERENTIATION AS REVEALED THROUGH STUDIES OF WHITE-SPOTTING PATTERNS IN MICE. THOMAS C. MAYER (Rider College, Trenton, N.J. 08602)
Embryonic skin and neural crest from mouse embryos of genotypes which produce white patches of fur on the coat (spotting) were tested for their pigment promoting or pig-

DOPA-POSITIVE NUCLEI AND MELANOGNESIS. HARriet M. McCURDy (University of Victoria, B.C., Canada)
Observations in this laboratory showed that in the California newt, Taricha torosa, the nucleus as well as the cytoplasm, was dopa-positive in the recognizable melanoblast. Earlier stages revealed a dopa-positive nuclei with no cytoplasmic reaction. This prompted a similar examination of the early development in 2 other urodeles, Ambystoma gracile and Taricha granulosa. In the former, the ectoderm of the blastula, stages 11-12 (Harrison) contained dopa-positive nuclei. This response faded by stage 15. During the closure of the neural tube, ectodermal nuclei again gave a dopa response. The melanoblast nucleus and cytoplasm of both A. gracile and T. granulosa were dopa-positive. This response was strongly marked in T. granulosa as the melanoblasts migrated to their position under the basement membrane. Again, the mature melanocyte showed no dopa response in either nucleus or cytoplasm. Intermittency of eumelanin-forming response in the melanocyte cytoplasm has been recorded by Laidlaw, Cleffman, Galbraith and others. The findings here presented show an intermittent response to exogenous dopa in the nuclei of two urodele embryos. Possible correlation of these findings with the nuclei of melanotic melanocytes is being explored.

ROLE OF MELANOCYTES IN RESPONSES OF THE SKIN OF MONGOLIAN GERBILS TO CHEMICAL CARCINOGENS. C. J. MCDONALD, W. C. QUevedo, Jr., T. C. BIENERI AND N. FauTo (Brown University, Providence, R.I. 02912)
The application of 7,12-dimethylbenz(a)anthracene (DMBA) either alone or in combination with croton oil to the hairy (trunk) skin of adult Mongolian gerbils (Meriones Unguiculatus) elicits striking pigmentary changes which are frequently associated with developing neoplasms. Croton oil alone produces progressive hyperpigmentation that results from a marked increase in active dermal melanocytes and from accumulation of melanin within dermal macrophages. No active melanocytes are observed in the overlying thickened epidermis. Unlike the croton oil-treated group, papillomas and squamous cell carcinomas developed in gerbils treated either with DMBA alone or in combination with croton oil. Melanocytes were often found in the dermis beneath papilomas, less frequently in the hyperplastic epithelium of papillomas, and occasionally in squamous cell carcinomas. In addition, many melanogenic epidermal melanocytes were found in the thickened non-papillomatous painted epidermis. As in the croton oil-treated animals, there was a striking increase in the number of cells of kind found containing macrophages. The source of melanogenic melanocytes in the dermis and epidermis of skin treated with chemical carcinogens remains to be established. Active melanocytes are present in the epidermis at birth, but disappear during the first month of life. The hyperplasia of dermal melanocytes may involve proliferation of melanocytes which usually form perifollicu-
THE CLASSIFICATION OF MELANOMA AND ITS RELATIONSHIP WITH PROGNOSIS. VINCENT J. MCGOVERN
(Fairfax Institute of Pathology, Royal Prince Alfred Hospital, Camperdown, New South Wales, 2050, Australia)
Melanoma is classified as originating in 3 ways. It can start in Hutchinson's melanotic freckle, or begin as a Pagetoid invasion of the epidermis (premalignant melanosis), or it can grow in nodular form without any initial spread laterally. In addition, the depth of invasion, the amount of pigment production, the presence of solar change in the dermis and the lymphocytic response of the tissues have been recorded. An attempt has been made also, to grade the degree of anaplasia of the component cells. These features have been analyzed in 202 primary melanomas examined in the 5-year period 1962-1965 in order to determine their influence on prognosis. The most important prognostic features seem to be the type of tumor and the depth of invasion. Nodular melanoma has the least favorable prognosis while melanoma commencing in Hutchinson's freckle seldom metastasizes. The deeper a melanoma penetrates the worse the prognosis and the more anaplastic tumors have a worse prognosis than those composed of cells more closely resembling non-malignant nevus cells.

β RECEPTORS IN MELANOCYTES. JOSEPH McGUIGE
(Yale University Medical Center, New Haven, Conn. 06510)
Cyclic AMP (adenosine-3′,5′-monophosphate) mediates the effect of many hormones including the catecholamines, glucagon, ACTH, and TSH.

The formation of cyclic AMP from ATP is catalyzed by the enzyme cyclase. The hormone receptor is thought by Sutherland et al. ( Circulation 37: 279, 1968) to be an integral part of the cyclase system.

MSH, ACTH, caffeine and cyclic AMP darken frog skin. These observations suggest that cyclic AMP functions as a second messenger in the melanocyte. Recently (Abe et al., Endocrin. 44: 362, 1969), cyclic AMP has been shown to increase in frog skin after the administration of MSH, or ACTH, further establishing the central role of this nucleotide in the darkening process. In this report the effects of α and β adrenergic agents on frog skin color have been examined.

(1) Norepinephrine, an α-adrenergic agent, lightens frog skin previously darkened with MSH. (2) Isoproterenol, a β-adrenergic agent, darkens previously lightened frog skin; this effect is inhibited by propranolol, a β-blocker. These observations suggest that both α and β receptors are associated with the adenyl cyclase system of frog skin.

GENE CONTROL OF DIFFERENTIATION OF THE MOUSE MELANOCYTE SYSTEM. BEATRICE MINTZ (Institute for Cancer Research, Fox Chase, Philadelphia, Pa. 19111)
Cost color patterns in allogeneic mice (obtained experimentally by aggregating blastomeres from genetically different embryos) reveal a melanocyte system with the following features. (1) There are 34 dorsoventral transverse melanocyte clones (1 per side, including the tail), each descended by mitotic proliferation from one genetically determined melanoblast in the neural crest. (2) There are approximately 150-200 somite-derived transverse hair follicle clones (half per side) that develop independently of the melanoblast clones. (3) Melanoblast clonal phenotypes (e.g., black vs. brown) can be differentially modified, forming subclones, when melanoblasts enter hair follicles of different phenotypes (e.g., agouti vs. non-agouti). (4) Genetic determinations of primordial melanoblasts and of hair follicle primordial cells occur early, possibly in the day 5-7 embryonic period, and generally remain stabilized throughout cloning. (5) White-spotting genotypes are characterized by preprogrammed death of melanoblasts, as a normal event that occurs in the course of cloning. (6) The undisturbed or standard patterns of melanocyte and of hair follicle clonal development are achieved in certain genotypes; but in many others, derived patterns are produced by a variety of genotype-specific forms of clonal selection. (7) Phenotypic clonal heterogeneity, despite identity of cell genotypes, may be a commonplace in development, with orderly selective interactions between the variant clonal subpopulations leading to a total pattern, or metaclonal phenotype.

DENDRITIC CELL DYNAMICS IN PROGRESSIVE DEPIGMENTATION. YUTAKA MISHIMA AND HIBWA KAWASAKI (Wakayama Medical University, Wakayama, Japan 620; Wayne State University, Detroit, and Veterans Administration Hospital, Allen Park, Mich.)
Changes in dendritic cell population during progressive depigmentation occurring in vitiligo vulgaris, Sutton’s leucoderma, and progressing Dubreuilh’s melanosis have been studied quantitatively by electron microscopy. In all 3 types of lesions melanocytes having premelanosome synthesis and tyrosinase activity show a sharp decrease or disappearance, while no significant decrease of Langerhans cells in the total epidermis is seen. However, it is found that junctional Langerhans cell population is characterized by over 100%. Furthermore, the decrease in melanocytes there is a proportionate increase, throughout the epidermis, of non-keratinocytic cells (α-cells) which have neither premelanosomes nor Langerhans granules. (Supported by NIH CA-88891 and Ministry of Education, Japan.)

MELANOCYTES IN AUSTRALIAN ABORIGINAL SKIN. R. E. MITCHELL (University of Tasmania, Hobart, 7001, Australia)
The Australian Aborigine belongs to a deeply pigmented race which is believed to have been isolated in Australia for over 10,000 years. A study has been made of forearm skin in 6 pure blood Australian Aboriginal males, using electron microscopic techniques, and histologic techniques including the dopa incubation of tissue slices and split skin. The melanocytes were more strongly dopa-positive and were significantly more numerous than in the same region in Caucasian (white Australian) skin. The electron microscope showed that melanosomes in the melanocytes were much more common than the premelanosomes. Melanosomes tended to be very dense even when small, and they attained a greater maximum size than melanosomes in exposed skin in white Australians. In the keratinocytes melanin occurred in numerous discrete melanosomes, and it did not appear to be destroyed in keratinocyte lysosomes to the same extent as in Caucasians. The ultraviolet light filtering capacity of Aboriginal skin is great, for in spite of the high solar exposure of this skin there was no evidence of chronic solar dermatosis in the skin of Aborigines.

THE ULTRA STRUCTURAL LOCALIZATION OF DOPA-H IN AMPHIBIAN MELANOBLASTS GROWN IN VITRO. PAT G. MODEL (Albert Einstein College of Medicine, Bronx, N.Y. 10461)
The onset of differentiation in the pigment cell component of amphibian (Ambystoma mexicanum) neural crest tissue cultures is marked by the concomitant appearance of numerous premelanosomes, arrays of polyribosomes, and cisternae of rough-surfaced endoplasmic reticulum (rER). An extensive and apparently unchanged Golgi complex is present prior to and during melanogenesis. As development proceeds, the number of premelanosomes and melanosomes increases. Premelanosomes are found in all stages of development but the proportion of mature granules becomes progressively larger. Although
some newly formed granules may appear in association with either the Golgi or the rER, many are not associated with any particular subcellular structure. Culture media for treatment with dopa-H2S and studied by means of high resolution radioautography. The specific localization of radioactivity in young pigment granules indicates that this organelle is the site of melanogenesis in differentiating amphibian melanoblasts. (Supported by NIH grants NB 07512, NB 07674, and MH 06418.)

CLINICAL CHARACTERISTICS OF MALIGNANT MELANOMA RESPONSIVE TO AN IMIDAZOLE CARBAMIDE (DTIC). LARRY NATHANSON (New England Medical Center Hospital, Tufts University School of Medicine, and Eastern Cooperative Oncology Group, Boston, Mass. 02111)

DTIC is an unstable compound and must be stored frozen and given IV. It produces marked increase in survival in mice bearing leukemia L-1210. In tissue culture and in vivo murine systems, it is not a cell cycle stage specific agent, and a dose response relation is not demonstrable. A prospective randomized study of 2.0 or 4.5 mg/kg/d in 10 daily IV pulse doses was carried out in 50 patients with metastatic melanoma; 38 were evaluable. Toxicity included mild leukopenia, nausea, vomiting and rare hepatic dysfunction. 7 of 22 patients on 2.0 mg/kg, and 3 of 16 on 4.5 mg/kg drug therapy were observed to undergo objective responses. Duration of response was from 1 to more than 8 months. Mean survival was 12 weeks in nonsponging, and 35 weeks in responding patients. This 22% objective response rate is about twice that of alkylating agents. DTIC is the most effective chemotherapeutic agent currently known for control of metastatic melanoma.

SOME ASPECTS OF THE CONTROL OF MELANIZATION IN GOLDFISH. M. OBIKA, J. MATUMOTO (Keio University, Yokohama, Japan) and J. D. TAYLOR (Wayne State University, Detroit, Mich. 48102)

Previous investigation has indicated that the pigment granelle (pterinosome) of the erythrophores of red-scaled goldfish exhibits tyrosinase activity when isolated and assayed in vitro. Despite the presence of the enzyme, transformation of pre-existing erythrophores into melanophores has never been observed. Melanization of the skin in vivo is induced by various treatments. Electromicroscopic observation of the melanized skin suggests that the induction of melanization occurs in melanoblasts. Tyrosinase activity of bright-colored skin appears to be inhibited by at least 2 substances. The inhibiting effect is diminished by the addition of sulfhydryl blocking agents and by heat treatment. Disintegration of isolated pterinosomes seems to be necessary for enzyme activation since intact pterinosomes exhibit very low tyrosinase activity. In sonicated pterinosomes, about 60% of the total enzyme activity is found in the membranous fraction. The goldfish enzyme oxidizes dopa rapidly, but it oxidizes tyrosine very slowly and only in the presence of a primer.

TYROSINASE ACTIVITY MEDIATED BY PEROXIDASE IN MAST CELLS, EOSINOPHILS AND MELANOMA CELLS. M. OKUN, L. EDELSTEIN, G. HAMADA, N. OBI, J. BURNETT and G. BLUMENTHAL (Dermatology Lab., Boston City Hospital, 818 Harrison Ave., Boston, Mass. 02118)

Histochemical studies in our laboratory have demonstrated tyrosinase activity in mast cells and eosinophils. Preincubation with sodium diethylidithiocarbamate (DCC) produced no inhibition, suggesting that inhibition with DDC in previous experiments was related to its antiodiiodid reaction rather than copper-chelating properties. Evidence that this tyrosinase activity was mediated by peroxidase was its suppression by catechol. Peroxidase-mediated tyrosinase activity was always correlated with a positive benzidine reaction. Peroxidase-mediated tyrosinase activity was also observed in HP melanoma cells. Epidermal melanocytes studied and other HP melanoma cells had catecholase-negative, and showed no evidence of cresolase activity, even in the presence of catalytic dopa. A substance having the absorption characteristics of dopachrome formed when isolated mammalian peroxidase was incubated with tyrosine in the presence of hydrogen peroxide and catalytic dopa. These preliminary studies suggest that peroxidase, rather than a copper-dependent phenol oxidase, may be the principal mediator of tyrosine hydroxylation in melanocytes and some non-melanocytes. (Gen. Res. Support Fund, BCH and St. Vincent Hosps. NIH Gr. T1 AM 3220 06 and CA 06292).


The augmentation of melanin pigmentation in human skin by solar radiation involves a close interaction of melanocytes and keratinocytes and is characterized by the formation of new melanosomes within the melanocytes and their transfer and distribution within an increased population of keratinocytes. This augmentation of melanin pigmentation revealed: (1) an immediate pigment-darkening oxidation reaction in the melanin present in already existing melanosomes and involves the formation of semiquinone-like free radicals; perinuclear redistribution of melanosomes in the basal and suprabasal cells; (2) an increase in the number of functional melanosomes as the result of proliferation and/or activation of melanocytes; (3) hypertrophy of melanosomes and increased arborization of their dendrites; (4) an increase in the number of melanosomes in all stages (early, intermediate, and fully melanized) both in the melanocytes and Malpighian cells due to increased synthesis of new melanosomes in melanocytes; (5) an increase in the number and size of melanosomes complexes in certain races; (6) an increase in the transfer of melanosomes as a result of increased turnover of keratinocytes; (7) an increase in tyrosinase activity due to the synthesis of new tyrosinase in proliferating melanocytes; (8) activation of tyrosinase as the result of the direct effect of radiation on tyrosinase-inhibiting sulfhydryl compounds.

IS WOLFF'S UNICELLULAR SEBACEOUS GLAND A MELANOCYTE? HISTOCHEMICAL AND ELECTRON MICROSCOPICAL OBSERVATIONS. C. PELFINI, S. SACCHI AND F. SERRI (University of Pavia, Italy)

In 1951 Wolff stated "There can be no reasonable doubt that unicellular sebaceous glands occur normally in the human epidermis." (Lancet 4: 888, 1951; Brit. J. Dermat. 63: 296, 1951). We have studied this problem in biopsies from palpebral epidermis, focusing upon the characteristic features of these cells, which are located in the basal layer of the epidermis, have no desmosome, and are dopa-positive. The cytoplasm is full of lipids which are collected in small droplets positive with Sudan, with Oil Red O and with the Weber reaction for cholesterolides.

Electron microscopy shows these cells to be round and protruding into the underlying dermis. The cytoplasm lacks tonofilaments and is full of large vacuoles which remain after the extraction of cholesterolides. In the cytoplasm there are pre-melanosomes and many free melanin granules which are never collected into melanosome complexes. The hypertrophic Golgi apparatus, the abundant endoplasmic reticulum, both smooth and rough, and the number of free ribosomes suggest that these cells are in full metabolic secretive activity. Keeping in mind all these observations, we think that these cells are either melanocytes which secrete or metabolize cholesterolides or cells with a characteristic individuality and the potential of synthesizing both melanin and lipid substances.

REGULATION OF MELANOPHORE CELL DIVISION IN XENOPUS LAEVIS LARVAE. F. W. PELLMANN (Anatomisches Institut der Universitat Kiel, Germany)

The increase in number of dermal melanophores of Xenopus...
A study has been made of the response of melanocytes and melanoblasts to X-rays and Actinomycin D, separately and in combination. The sensitivity appears to depend greatly on the stage of the melanoblast cycle at the time of treatment. Telogen Go melanoblasts are killed by X-rays as shown by the melanoblast survival curve (N = 6, D0 = 200 rads). Division transitionable 3 days after plucking, seem to have a slightly different sensitivity, as defined by Do, but show a large increase in the value of the extrapolation number, N. This increase is not attributable entirely to the increased number of melanoblasts. Irradiation of functional melanocytes, 9 days after plucking, results in considerable apparent increase in the number of melanocytes per follicle, many being abnormally located. These changes are concomitant with gross changes in the follicle. Actinomycin D has little or no apparent effect on Go amelanotic melanoblasts, when judged by the same criterion as the X-ray response. The effect of functional day 9 melanocytes is morphologically identical with that for X-rays. For both agents this day 9 response is very sensitive. The Actinomycin D day 3 response appears to be intermediate between the telogen and day 9 reaction. The effect of combining X-rays and Actinomycin D appears to be synergistic, at least for treatments in telogen.

**HUMAN PARTIAL ALBINISM: AN ELECTRON MICROSCOPE STUDY.** Michel Prenieras (Fondation Ad. de Rothschild, Laboratoires de Recherches, Paris, France)

In recent years, evidence has accumulated to support the theory that Langerhans’ cells and melanocytes represent 2 different cell populations. However, at least one puzzling question remains: the "replacement" of melanocytes by Langerhans’ cells in human partial albinism, whether acquired (vitriligo) or hereditary (piebaldism). Our present studies have examined the structure of melanocytes in human partial albinism, comparing normal skin, depigmented areas, and transitional zones where depigmentation is underway. Two cases of piebaldism, 12 cases of vitiligo, and 2 Sutton’s Noevi have been studied. Electron micrographs suggesting that Langerhans’ cells may have a damaging effect upon melanocytes have been examined. Direct elimination of melanocytes by Langerhans’ cells is proposed as a working hypothesis to explain some partial hypopigmentations in humans, both hereditary and acquired.

**ADRENERGIC INNERVATION OF MELANO PHORES IN A TELEOST FISH.** B. L. Reed and B. C. Finnin (Victorian College of Pharmacy, Parkville, Victoria 3052, Australia)

A sensitive method for the continuous recording of melanophore responses in vivo has been developed. The technique involves measuring light transmitted through the fin of a spinal-sectioned fish and allows the study of small or rapid

**PIGMENT CELL CONFERENCE ABSTRACTS**

**GREAT INTERACTION AND HAIR PIGMENTATION IN THE MOUSE, L. J. Pierro and J. Spiegel (University of Connecticut, Storrs, Conn. 06268)**

Size, shape and number of eumelanin granulas are affected by the beige (bg) mutation (formerly slate, J. Hered. 54: 47, 1963). Fewer granules are synthesized by melanocytes, and the number is further reduced by fusion of granules into larger pigmented bodies. Pigment granules are present in both cortex and medulla of the hair; medullary granules tend to clump together.

Previous studies have shown that interaction between bg and either p or ru affect the distribution of granules within the hair shaft; cortical pigment is absent in the double homozygote. Genic interactions involving bg can also affect the distribution of granules along the length of the hair shaft. Exclusion of eumelanin pigment from the proximal region of the hair shaft, a feature characteristic of the B11 mutation, is not observed in mice also homozygous for bg. Phaeomelanin, on the other hand, may be eliminated from the proximal hair shaft in yellow (A y/a) mice homozygous for bg. This latter interaction occurred during later hair growth cycles in a small percentage of animals in an unselected stock. Selection for an increased increase in hair was made first with a lightening of the coat after completion of the second hair growth cycle, and subsequently with the absence of phaeomelanin from the proximal hair shafts produced during the first hair growth cycle. Melanocytes appear to be present in the hair follicles throughout the cycle. (Supported in part by PHS Grant N607198–05.)

**PITUITARY SEROTONIN CONTENT AND THE CONTROL OF MSH SECRETION.** Ramon Pizziri and Richard Wurtman (Massachusetts Institute of Technology, Cambridge, Mass. 02139)

It has previously been shown that intraperitoneal injections of melanin liberate the melanocyte-stimulating hormone (MSH) from the rat pituitary gland. Treated animals show changes in brain serotonin, and it has been shown that this effect is mediated by the pituitary. Preliminary studies have shown that the more rapid changes in brain serotonin and ACTH are observed within 30 minutes after injection of MSH. This effect is dose-dependent and occurs after injection of MSH to both male and female rats. The mechanism of this effect is under study.

**THE VARIATION IN SENSITIVITY TO X-RAYS AND ACTINOMYCIN D OF MELANOCYTES OF DIFFERENT PHYSIOLOGICAL STATES, C. S. Potten (Allegheny General Hospital, Pittsburgh, Penn. 15212)**

*gers*, observed in a special area of the skin of the head between stages 52/53 and 59, takes place by cell divisions (80%) as well as by new differentiations (20%). Experimental conditions can change the total increase and also the relation between cell divisions and new differentiations. Hypophysectomy stops both divisions and differentiations. Implantation of a pituitary induces an extremely high increase of melanophore divisions (34%). Animals housed in white containers show a decrease in differentiations, down to 12%. Animals in permanent darkness exhibit an increase in differentiations up to 70%, whereas the total melanophore increase remains normal. Areas of different melanophore density (2–125 Mel/mm²) were marked out on the dorsal side of the head and over the muscular system of the tail. Animals on a white background show a decrease of melanophores in these areas down to 5–50%. An increase of MSH activity is followed by an increase of melanophores up to 155–3700%. In larvae hypophysec tomized for several weeks, there is an increase in melanoblasts in special areas, as a function of time of day. Melanocytes appear to be present in the hair follicles throughout the cycle. (Supported in part by PHS Grant N607198–05.)

**STRUCTURE AND BIOGENESIS OF PHEOMELANINS.** G. Prota (Univ. of Naples, Italy)

Pheomelanins are alkali-soluble pigments which as yet have been found only in hair and feathers. Chemical and biochemical studies of the pigments from New Hampshire chicken feathers give conclusive evidence that pheomelanins are formed in vivo by a deviation of the eumelanin pathway involving a reaction between cysteine and dopaquinone, produced by enzymatic oxidation of tyrosine. Moreover, it has been shown that this reaction leads mainly to the formation of g-(5- cysteiny1-3,4-dihydroxyphenyl) alanine, whose further oxidation, probably by dopaquinone itself, gives rise to pheomelans.

Evidence is also presented which suggests that the so-called trichosiderins, the red pigments of red human hair, are chemically and biogenetically related to pheomelanins. This view is supported by the structure of a yellow-orange pigment, CalsHnN105, extracted from the feathers of New Hampshire chickens.

**THE VARIATION IN SENSITIVITY TO X-RAYS AND ACTINOMYCIN D OF MELANOCYTES OF DIFFERENT PHYSIOLOGICAL STATES, C. S. Potten (Allegheny General Hospital, Pittsburgh, Penn. 15212)**
melanophore responses which could not be detected by other means.

Responses to electrical stimulation of the spinal cord and to injected catecholamines and antiderenaline drugs have been investigated using the fresh-water teleost, Pterophyllum

c. The results indicate that the melanophores of this fish are innervated by sympathetic fibers and that the melanophores appear to be aggre-

gated and beta-adrenergic receptors that mediate pigment disper-

s. These findings probably explain the results of Speth (1916) and later studies by who reported that ergotamine (a drug which blocks adrenergic receptors) caused reversal of the normal melanophore response to electrical stimulation so that stimulation produced pigment dispersal instead of aggre-

gation. Studies with parasympathetic drugs and their antagonists show that the parasympathetic nervous system does not play a role in the control of melanophores in P. c. eimekei.

STUDIES OF NORMAL MELANOCYTES IN CULTURE. P. A. Rule (University College Hospital Medical School, London, G.1, England)

Normal melanocytes from an inbred strain of black guinea pigs have been isolated by a method based on that of Prunieras (Arch. Derm. 8: 23) and grown in microultire cultures in Cruikshank chambers. Observations on the behavior of the cultured cells have shown them to be remarkably immobile, particularly when aggregated in clonal origin. This suggeststhat movement by a highly favored energetic relationship between the cells, and with the glass substrate, single cells show much greater tendency to move about and make exploratory contacts with other cells.

Nucleoside polyphosphatase (NPPase) activity of the type previously ascribed to a myosin-like ATPase (J. Invest. Derm. 47: 412) can be demonstrated in the motile but not in the stationary cells. In mixed cultures strong NPPase activity is found in keratinocytes which show very considerable surface movement. These data strongly support the view that a direct relationship exists between the amount of movement at the cell surface and the degree of NPPase activity. Autoradiographic studies of cultured melanocytes labelled with 3H-thymidine have shown that a relationship also exists between the degree of cell motility and the incorporation of thymidine into nuclear DNA. This finding would seem to support the hy-

thesis of Carter (Nature 220: 970) that the motility of a cell is related to its surface energy conditions.

EFFECTS OF FOUR RADIATION-INDUCED LETHAL ALLELES AT THE ALBINO LOCUS ON THE FINE STRUCTURE OF MELANIN GRANULES IN THE MOUSE. ELIZABETH RITTENHOUSE (Albert Einstein College of Medicine, Bronx, N.Y. 10461)

The e locus in the mouse is commonly assumed to be the structural locus for tyrosinase, but radiation-induced recessive lethal alleles at the locus are known to cause not only albinism but also deficiency of glucose-6-phosphatase. Such mutations could be interpreted as deletions or as evidence sug-

gesting that the e locus may control some function impor-

tant for the activity of both enzymes. Embryonic retinal pigment epithelium was examined with the electron microscope to determine the effect of these alleles on melanin granule structure. In each case pigment cells of albino homozygotes contained bodies recognizable as unmelanized granules. In the embryos, 3 alleles produced granules obviously abnormal in number, size, and shape. Granule structure in the radiation-induced mutants is compared with granule structure in viable albino and the implications about the nature of the alleles discussed.

ESTABLISHMENT AND CHARACTERIZATION OF HUMAN MALIGNANT MELANOMA CELL LINES GROWN IN VITRO. M. M. ROMBAHL AND T. C. HSU (University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77023)

Eight human malignant melanoma cell lines from different patients have been established, 2 being propagated for over 4 years. The lines have a dendritic, cuboid, or spindle morphol-

ogy, are variable in regard to pigment formation, and have characteristic mesodermal between 43 and 78. These features have
generally remained stable under in vitro conditions.

Cloning of 2 lines has yielded strains which have a specific morphology, growth pattern, and pigment content. The chro-

mosome number of several strains were uniform while others had a greatly restricted range as compared to the parent line. Karyotypes of cells within a strain were similar although different between strains. Marker chromosomes were often observed. Cell doubling times of cloned strains varied from 25 to 45 hours. Tyrosinase activity, determined fluorometrically, varied widely among the strains. Experiments suggest an in-

verse relation between in vitro growth and pigment production. These studies indicate that human malignant melanoma tu-

mors may be composed of discrete, stable cell populations which differ widely in growth, potential, structure and function.

ULTRASTRUCTURAL IDENTIFICATION OF MELANO-

CYTES IN EARLY HUMAN EMBRYOS. R. W. SAGERIEL AND G. F. OLAND (University of Washington School of Medicine, Seattle, Wash. 98105)

Cells of the melanocyte system have been identified using the light microscope in the dermis of embryos of 10-12 weeks of age and in the epidermis of 12-14 week fetuses. Ultrastru-

tural observations have demonstrated melanocytes in the epi-

dermis of 12 and 14 week human fetuses. The present observa-

tions report melanocytes in the epidermis of 3 human embryos of 8, 8, and 10 weeks gestational age. The melanocytes present within these cells resembled adult melanosomes, but were smaller in size, measuring approximately .15 to .4 µ. In the older specimen early melanin pigment appeared to be de-

posited on the melanosome, whereas the melanosomes in the younger embryos were not melanized. These observations narrow the interval between the time when the neural crest has com-

pleted its differentiation and the time when melanin pro-

ducing cells can be identified in the epidermis. (Supported by USPHS Grant CA 10522-02 and USPHS Grant AM 09385.)

COMPARATIVE EFFECTS OF PIEBALD SPOTTING GENES ON CLONES OF MELANOCYTES IN DIFFER-

ENT VERTEBRATE SPECIES. R. H. SCHAIBLE (Indiana Univ., Bloomington, Ind. 47401)

Comparisons of mutant types with the standard (normal or wild) type within a species are commonly used to elucidate the steps at which genes control developmental processes. Comparative studies among species can accomplish the same end, providing that the loci involved can be shown to be homologous either by descent, or by their control of physio-

logical effects. Piebald-spotted animals typically show pig-

mented spots on a white background. The locations of the spots are usually consistent among animals of the same species. The pigmented spots vary in size to the extent that complete absence occurs at one extreme and merger of adjacent spots occurs at the other extreme. Each spot is formed by the proliferation of a clone of melanocytes from a single primordial melanoblast. Comparisons among piebald-spotted mutants within and among vertebrate species, including man, indicate that the locations of the primordial melanoblasts and the sizes of the clones are genetically established. Certain interpreta-

tions concerning the location of the primordial melanoblasts and mechanisms by which genes control development of pig-

ment cell clones have been proposed as a result of recent studies exclusively on the mouse. Comparative studies among species indicate that alternate interpretations may be more valid.
THE ACTION OF PSYCHOACTIVE DRUGS ON PIGMENT CELLS OF LOWER VERTEBRATES. GEORGE T. Scott (Oberlin College, Oberlin, Ohio 44074)

In endocrine-controlled melanophores, as found in the frog, the skate, phenothiazine tranquillizing drugs are potent agents in causing darkening of the animal. Since these pigment cells are refractory to the drugs after hypophysectomy, the action of these tranquillizers is presumably upon brain centers controlling the release of pituitary intermedin. Anti-depressant drugs, on the other hand, suppress melanophore pigment dispersion. However, in the flatfish Scophthalmus, the action of such drugs is at the neuro-chromatophore junction, where evidence implicates a catecholamine. Phenothiazines were found to be the most potent of all pigment-dispersing agents. Four of the 12 phenothiazines are especially active. Pretreatment with catechol-o-methyl transferase (COMT) inhibitors raises the effective dose of these four drugs drastically, but has no effect on the other phenothiazines. A similar response to COMT inhibitors was found with alpha-adrenergic blocking agents, on the other phenothiazines. A similar response to the 12 phenothiazines are especially active. Relative activities are correlated with chemical structure.

TYROSINASE IN MEMBRANE SYSTEM OF MOUSE MELANOMA. M. Seki, H. Itakura, K. Miyazaki and T. Imaizumi (School of Med., Tokyo Med. and Dent. Univ, No. 1-5-45, Yushima, Bunkyo-ku, Tokyo, Japan)

Tyrosinase is located not only in the melanosomes but also in the smooth-surfaced membrane (SM) and in the rough-surfaced membrane (RM) isolated from mouse melanoma. It has been thought that the SM is a precursor of the melanosome. The state of tyrosinase in such biological membranes was studied in order to clarify the biochemical process of melanosome formation.

The SM isolated from mouse melanoma was treated with Naja Naja venom phospholipase A. The amount of tyrosinase activity solubilized was proportional to the amount of protein in the supernatant and the specific activity in the supernatant and sediment was almost the same. Most of tyrosinase activity remained in the residue of digested membranes. When the SM was digested with protease, 60-70% of the total tyrosinase activity was solubilized in the supernatant and when tyrosinase was isolated through gel filtration, the specific activity of tyrosinase was 100 times as high as that of the SM. Electrophoretic pattern of soluble tyrosinase thus isolated was observed to consist of two components. The SM was purified from the RM by DOC treatment; the specific tyrosinase activity of the SM thus obtained was lower than that of the ordinary SM.

GENETICS OF PIGMENT CELL BEHAVIOR IN SHEEP. J. A. Serra (Pacific Northwest Research Foundation, Seattle, Wash. 98104)

When pigment cells enter or not, hair and fiber roots in large areas or the whole surface of the cost are determined in sheep by at least 1 dominant locus C-c and 1 semidominant L-l. Constitution C (C.c) ppm produces absence of pigment in the whole cost. N is for dominant pigmentation, N (Nw) - C (C.c) producing pigmented cost, and pp is for recessive pigmentation. Both C and L permit melanocyte activity in eyes and skin. Another locus, J-j, determines melanocyte behavior at the level of individual hair and fiber roots; jj sheep show a mixture of white and pigmented fibers at birth or whitening progresses until about 6 months of age. In fibers during depigmentation, melanocytes migrate from root to follicle, which then shows more melanocytes than usual. Other melanocytes are phagocytized as in the autoimmune reaction. Depigmentation may also take place with 1 dose of j and 1 of C or L in the presence of N. Two doses of J and 1 of C with N determine a coat which may be entirely white or almost so except for pigmented coarse fibers or hairs. Different alleles determine quantitative differences in depigmentation. Thus, melanocyte behavior in entering or leaving the fiber roots and undergoing an autoimmune reaction is determined in sheep by at least 3 loci and by the type of hair growth, whether for a fine fiber or a coarse hair.

EFFECTS OF CARCINOGENS ON HUMAN EPIDERMAL PIGMENT CELLS IN VITRO. F. Serri and G. Pisanu (University of Pavia, Italy)

The effect of 9,10-dimethylbenzanthracene (DMBA) and other carcinogens on human epidermal pigment cells have been studied in vitro. These drugs, in varying concentrations, were added to the cell cultures after 4-7 days.

Preliminary results show that DMBA at the concentration of 0.001 µg/cc does not produce any immediate toxic effect. At concentrations from 0.01 to 10 µg/cc, selective destruction of melanocytes is observed after 24-48 hours, while keratinocytes are not affected. Higher concentrations cause the immediate destruction of melanocytes, while keratinocytes are altered, but at a slower rate.

CONTROL OF PIGMENT PRODUCTION IN SYNCHRONIZED MELANOMA CELLS IN VITRO. S. Silagi (Cornell University Medical College, New York, N.Y. 10021)

A clonally derived amelanotic melanoma cell line has repeatedly been forced to produce pigment by the inhibitor of DNA synthesis 1 x 10^-3 D-arabinofuranosylcytosine (ara-C) at sublethal levels. One ara-C derived melanotic line has been cloned, and has continued to produce pigment for 2 years on normal medium. The inhibitor is most effective when administered to synchronized cells in 4 pulses on successive days at 1.5 x 10^-5 M during the S phase of the cell cycle. Colcemid at a sublethal concentration, and growth on medium solidified with agar also evoked pigment production in this line, but a large number of other inhibitors of biosynthetic processes did not, under the conditions tested. The melanotic lines are active producers of tyrosinase (dopa oxidase), whereas the amelanotic line produces an inhibitor of tyrosinase activity. Both enzyme and inhibitor are labile at 4°C and -20°C, and decay of the inhibitor in homogenates of amelanotic cells reveals a low level of residual dopa oxidase activity. The mean population doubling time of a cloned melanotic line is 23 hr. and that of a cloned amelanotic line is 16.5 hr. This difference in rate of growth is believed to be a significant factor in maintaining this differentiated function and may also account for the production of an inhibitor by the rapidly dividing amelanotic cells. (Supported by Grant CA-10065, National Cancer Institute, USPHS.)

MELANOBlasts IN THE ADULT HAIR FOLLICLE GERM, AND THEIR CONVERSION TO MELANOcyTES. A. F. Silver and H. B. Chase (Brown University, Providence, R.I. 02912)

Isolated dendritic clear cells (melanoblasts) are located in the germ of the resting (telogen) hair follicle, touching the basement membrane between germ and dermal papilla. During telogen they are small, smoothly rounded, with inconspicuous chromatin and cell organelles. After spontaneous or experimental activation of the hair germ, they enlarge, chromat in and cell organelles become evident, they divide repeatedly, and produce visible pigment during anagen III-IV, always remaining in the same position relative to the dermal papilla.

At the end of anagen VI the melanocytes have ceased to make pigment. They regress and, during catagen, only a few altered pigment cells, next to the dermal papilla, are recognizable. These are retained in the hair germ as dormant melanoblasts during the subsequent resting stage. (Supported by USPHS Grants FR-07085-03 and CA-06592-18.)
HORMONAL CONTROL OF HAIR COLOR. RICHARD SWELL (New Jersey College of Medicine and Dentistry, Jersey City, N.J. 07304)

All red or all black mature virgin female guinea pigs were used. They were not genetically pure strains. Hair samples were obtained from the scalp and anterior abdominal wall of each animal before and during the course of each experiment. Changes in hair color were assessed by (1) objectively comparing the numbers of hairs of the same color in the different samples and (2) subjectively comparing the overall color of the different hair samples.

Group I: Hair samples were removed at monthly intervals from 12 untreated control animals. Group II: Hair samples were removed from 12 animals before ovariotomy, 1 month after ovariotomy and 1 month after receiving estradiol benzoate (0.1 mg per day intramuscularly). Group III: Hair samples were removed from 9 animals before copulation, the 4th week of pregnancy, 8th week of pregnancy and immediately following parturition.

The results showed that ovariotomy, estrogen and pregnancy have no definite effect on hair color. This lack of response of the follicular melanocytes to the procedures in the present experiments is in marked contrast to the response obtained previously with the epidermal melanocytes; the latter are inhibited by ovariotomy and strongly stimulated by estrogen and pregnancy. The significance of the independent activity of morphologically identical pigment cells will be discussed.

FURTHER STUDIES ON THE STRUCTURE OF DOPA MELANIN. G. A. SWAN (Univ. of Newcastle Upon Tyne, England)

Previous studies have been described in which samples of (±)-dopa were labeled to the extent of 80% at (a) the α-hydrogen atom, and (b) both β-hydrogen atoms were prepared and converted into melamins (i) by autoxidation and (ii) enzymatically. These studies provided information as to the extent of linkage at the 2- and 3- positions of indole-5,6-quinone units in the polymers. We have now completed a more extensive investigation to obtain information on the extent of coupling in the 4- and 7- positions of indole-5,6-quinone, and also on the relative proportions of uncyclised (dopa) and cyclised (indo) units. Samples of (±)-dopa labeled with deuterium at the 4- and 7-positions, respectively, were prepared and converted into melamins (i) by autoxidation and (ii) enzymatically. The enrichment of deuterium in the polymers before and after treatment with acid was measured. [Carboxyl-14C]-dopa was converted into melamins (i) by autoxidation and (ii) enzymatically. The radioactivities of the resulting melamins were measured; and again after acid treatment or thermal decarboxylation. An oxidative degradation to yield a radioactive pyrroline-2,3,5-tricarboxylic acid was also achieved. These results provided information as to the relative numbers of uncyclised units, and cyclised units still containing a carboxyl group (dopachrome, pyrrole-carboxylic acid units, etc.). The general picture which emerges is one of an irregular polymer, containing several different types of units, linked in a number of different ways.


The formation of melanosomes in the melanocytes and the distribution of melanosomes inside keratocytes varies in the major ethnic groups (Caucasoid, Mongoloid and Negroid). The development of melanosomes in melanocytes shows the following differences: In a pale Caucasoid, unmelanized melanosomes (Stage I-III) are present; the melanized melanosomes are transferred into the keratocytes; in a dark Caucasoid, Mongoloid and Negroid, the number of melanosomes in Stages I-III is lower, whereas the number of melanized melanosomes is higher. The most significant racial difference between Caucasoids and Mongoloids on one hand, and Negroids on the other, is a difference in the distribution of melanosomes inside the keratocytes. In the Caucasoids and Mongoloids, the melanosomes are grouped in melanosome complexes. In the Negroids, however, the melanosomes are usually not grouped but are dispersed individually inside the keratocytes. These results suggest that racial differences in human coloration are related to the differences in the formation, maturation and distribution of melanosomes. The racial differences in the melanosome dispersion inside the keratocytes indicates that the macroscopically visible color variation may be partly due to the fact that in some races large melanosome complexes are present, whereas in others the melanin is dispersed in the very fine form of individual melanosomes.

REGULATORY FUNCTION OF THE AGOUTI LOCUS IN THE MOUSE MELANOCYTE. TAKUMI TAKUSHI (Miyagi College of Education, Sendai, Japan 980)

The a locus is responsible for the agouti pattern which involves the alteration of two types of melanin formed in the melanocytes of the hair bulbs. When a piece of dorsal skin from a 2-day-old mouse homozygous for the A allele was cultured in vitro, no yellow pigment (phaeomelanin) was recognized in the hair bulbs. On the other hand, the formation of phaeomelanin was observed when a skin explant from a 3-day-old mouse was cultured for 4 days. Addition of dopa to the culture medium of 2-day-old mouse skin resulted in the formation of phaeomelanin. The culture medium with excess tyrosine, on the other hand, inhibited phaeomelanin formation in the explant from a 3-day-old mouse. A regulatory system in the formation of agouti may be involved in the development of the agouti pattern in mouse hair.

SOME ASPECTS OF IRIDOPHORE REFLECTING PLATELETS. JOHN D. TAYLOR (Wayne State University, Detroit, Mich. 48202)

Amphibian and reptilian iridophores have been examined by electron microscopy. They are characterized by a number of structures which represent the former presence of their pigmented organelles, reflecting platelets. These are found either arranged in stacks or rows. Each reflecting platelet space is surrounded by a double membrane system. The space may contain phaeomelanin or melanosomes, or in some cases may be empty. Such platelets are scattered throughout the cell. The membranes respond to intermedin by migrating with the melanosomes from the perinuclear region into the melanophore processes. Their response at the organellar level includes condensation of material at their centers and ultimate disappearance. The possibility that these structures are reflecting platelets is discussed. (Supported by Grants GF-278 and GB-3681 from NSF.)


We have previously reported preliminary results on the pigmentation characteristics of 2 cloned cell lines from B16 melanoma. One of these is heavily pigmented while the other is
very lightly pigmented. Further studies have now shown that the pigmentation of these and other cell lines change fairly rapidly upon successive serial propagation in liquid culture. Studies with isolated clones of cells indicate that in a population of heavily pigmented cells, a significant fraction (more than one cell per 10,000) of cells have pigmentation characteristics of an unpigmented cell line. Similarly, a fraction (more than one cell per 10,000) of cells from a population of “unpigmented cells” have pigmentation characteristics of a heavily pigmented cell line. The unpigmented lines are much more sensitive to UV-irradiation than the pigmented lines and UV-irradiation can be used as a selecting agent for pigmented lines. Tyrosinase activity of cultures may be (1) not detectable (for unpigmented cell lines), (2) parallel to the growth curve or (3) greatly increased when growth stops.

X-RAY DIFFRACTION STUDIES ON MELANINS. Y. T. Thathachari (Stanford University School of Medicine, Stanford, Calif. 94305)

X-ray diffraction studies on a series of natural and synthetic melanins have been carried out. The diffraction maximum was at 3.4 Å for all animal and synthetic melanins, at 4.1 Å for ustilago melanin, and at slightly larger than 3.4 Å for plant melanins. Besides a sharper maximum at 3.4 Å, phaeomelan showed peaks at 14 Å, 8.4 Å, and 5.9 Å. These data suggest that the adjacent planar groups in all except ustilago melanin tend to aggregate in nearly parallel stacks. The radial distribution curves derived from very precise data for some of these melanins are very similar and consistent with this model. These curves show prominent peaks at 3.0 Å and 4.6 Å that would correspond to first neighbor distances between nonbonded atoms in adjacent nearly parallel planar groups. Peaks at 1.4 Å and 2.5 Å evidently correspond to the first and second neighbor distances within the planar groups. In ustilago melanin the adjacent groups are probably randomly disposed. Computed diffraction curves based on this model are in agreement with the experimental curves.


The level of tyrosinase activity in ECRPE shows marked changes with development. The peak activity appears to be on the 10th day of development when there is the largest number of melanosomes, especially Stage I melanosomes. In contrast, after the 18th day of development almost no Stage I melanosomes are seen and there is no tyrosinase activity. Experiments to be reported suggest that the origin of the Stage I melanosome is the large vesicle in the Golgi area. Melanosomes were studied in isolated fractions and in tissue culture of ECRPE by biochemical techniques and electron microscope histochemistry and radioautography. The most intense specific tyrosinase activity is present in the Stage I melanosome. In tissue culture, dopa-ßH uptake is observed in Stages I and II. Tyrosinase activity as shown by electron microscope radioautography using tyrosine-ßH-dopa (10:1) was present in Stages I through IV. The acrylamide gel electrophoresis pattern of soluble tyrosinase from an ECRPE is also changing markedly with development. It is believed that the major action of tyrosinase occurs on the Stage I and II melanosomes, and that the melanin polymer becomes detectable in the Stage III and IV melanosomes because of continued nonenzymatic formation of melanin polymers.

A number of experiments on the analysis of the mechanism of pigment migration in the melanocytes of amphibians, point to adenosine 3',5'-cyclic monophosphonate (3',5'-AMP) as one of the mediating substances in this hormone regulated process. In this concept pigment dispersion is induced by increase in the 3',5'-AMP content, resulting from activation of adenylcyclase (AC), and/or inhibition of 3',5'-nucleotide phosphodiesterase (PDE). Pigment aggregation results from the reversed processes.

Using isolated tailfins of Xenopus laevis larvae, the dispersing action of MSH is inhibited by the ß-blocking agent Inderal. This inhibition corresponds with the ß-receptor properties ascribed to AC.

That not only AC but also PDE may be involved in the above-mentioned concept, is demonstrated by the dispersion induced by the PDE inhibitor theophylline. Moreover, the product of PDE action, 5'-AMP, demonstrates a strong aggregation of the pigment granules, previously dispersed in absolute darkness.

The role played by 3',5'-AMP in the physiological melanophore reaction, is shown by the fact that preliminary experiments reveal a larger amount of 3',5'-AMP in extracts of dispersed skins of adult animals than in aggregated ones.

ACTIVATION OF TYROSINASE BY CHLORPROMAZINE. Mervyn H. Van Woert (Yale Univ. School of Medicine, New Haven, Conn. 06510)

Since long-term chlorpromazine (CPZ) administration in man is associated with cutaneous hypopigmentation, the effect of phenothiazine compounds on tyrosinase and melanin synthesis was investigated.

In vivo, CPZ (10mg/kg/day) administered I.P. for 10 days to C3HBl mice bearing B-16 melanomas doubled the tyrosinase activity in the tumors and produced a greater than 2-fold increase in the incorporation of C14 labelled dihydroxyphenylalanine into the B-16 melanoma. In vitro, 10-3 M CPZ produced a 2-fold increase in B-16 melanoma tyrosinase and a 50% increase in Harding-Passey melanoma tyrosinase.

The per cent increase in enzyme activity produced by CPZ was 4 times greater in the melanosomal tyrosinase than in the tyrosinase located in the remainder of the cell.

Other phenothiazine compounds also activate melanoma tyrosinase. Substituents in position 2 in the phenothiazine ring showed the following order of potency for activating tyrosinase: CF3 > CI > H. At the 10 position of phenothiazine a pipеразинолуксил side chain was more effective in increasing tyrosinase activity than an aliphatic amine.

The activation of tyrosinase and increase in melanin synthesis produced by CPZ may be a factor in the CPZ-induced cutaneous hypopigmentation in man. (Supported by USPHS grant NB07342 and AEC Contact AT (30-1)3960.)

THE ACTION OF COLCHICINE AND SULFHYDRL REAGENTS ON TELEOST MELANOPHORES. Muriel A. Wikso and Roxana R. Novales (Northwestern University, Evanston, Ill. 60201).

Various drugs and hormones have been shown to have an effect on melanosome migration in melanophores. Microtubules have been observed in teleost melanophores and they have been implicated as having an influence on pigment migration. Colchicine and several sulfhydryl reagents known to break down microtubules have an effect on melanin movements in melanophores. Pretreatment of scale melanophores of Fundulus heteroclitus with colchicine, mersalyl, or mercaptophtanol results in a decrease in the rate of aggregation of the melanin in response to epinephrine. This effect depends upon the pretreatment time and concentration of the test substance. These agents also produce dispersion of melanin in punctate melanophores which is greater than that produced by Ringer’s alone.
Cysteine hydrochloride reverses the action of colchicine and mersalyl on the response of the melanophores to epinephrine. This indicates that the action of these agents involves a reaction with sulphydril groups. There are several possibilities as to the mode of action of these various agents. They might break down microtubules which in turn could be involved in melanin aggregation. Possibly they affect the viscosity of the cytoplasm bringing about solution and inhibiting the gelation which is necessary for or which accompanies aggregation of the pigment. It is also possible that they could be acting at the level of the epinephrine receptors. (Supported by NSF Grant GB-4956X.)

PARTIAL CHARACTERIZATION OF A NATURAL DOPA AUTOXIDATION INHIBITOR AND ITS MODE OF ACTION. P. F. WILDE AND P. C. FLAWN (Unilever Research Laboratory, Isleworth, England)

A natural inhibitor of dopa autoxidation has been isolated from whole guinea pig skin. Its molecular weight has been assessed by gel filtration to be approximately 6000. It is a heat stable, alkali labile protein which is able to inhibit the conversion of tyrosine to melanin by mushroom tyrosinase and also melanin biosynthesis from tyrosine by slices of guinea pig epidermis. Its inhibitory action depends upon the ability to irreversibly bind tyrosine and dopa making them unavailable for enzyme action.

HETEROGENEITY IN HUMAN ALBINISM. CARL J. WITKOP, JR., JAMES WHITE AND WALTER E. NANCE (Univ. Minn., Minneapolis, Minn. 55455, Univ. Ind., Indianapolis, Ind. 46208)

Although oculocutaneous albinism has been regarded as a classic example of an autosomal recessive trait in man attributable to a defect in the enzyme, tyrosinase, recent evidence indicates 2 general types. Genetic studies of 2 families, 1 Negro and the other Caucasian, wherein both parents exhibit generalized albinism but who have produced only pigmented children indicate 2 nonallelic mutations for albinism. Hair bulbs incubated in tyrosine and electronmicroscopic studies of melanocytes from parents indicated that in each family one parent had tyrosinase activity in melanosomes and the other melanosome from parents indicated that in each family one

The enzyme also appears within premelanosomes. The modes of intracellular acid phosphatase transfer within melanocytes and the biologic significance of this enzyme will be discussed.

AN ELECTRON MICROSCOPIC STUDY OF THE EFFECT OF ULTRAVIOLET IRRADIATION ON HUMAN SKIN: CELLULAR CHANGES AND MELANOSOME DEGRADATION IN MELANOCYTES. A. S. ZELICKSON, J. H. MOTTAE AND J. A. HUNTER (University of Minnesota Medical School, Minneapolis, Minn. 55455)

Direct counting of cells and linear scanning of electron micrographs to determine volume ratios for epidermal dendritic cells revealed an increase in melanocyte number and an essential absence of Langerhans cells following 2 weeks of daily UV irradiation to human skin. Melanosome complexes were increased in keratinocytes, melanophages and significantly in melanocytes. The complexes are present in greatest number and size 48 hours after irradiation. Cytochemical demonstration of acid phosphatase activity within the melanoctyes complexes and the subsequent demonstration of the destruction of the premelanosomes and melanosomes within these complexes strongly suggests that the melanocyte has autolytic capabilities and the ability to degrade pigment which has been formed within the cell. (Supported in part by USPHS Grant AM05560.)