Pigment Cell Res 1993; 6:127–133
Printed in the United States of America

PIGMENT CELL RESEARCH
ISSN 0893-5785

Interaction Between Chemicals and Melanin

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Various drugs and other chemicals, such as organic amines, metals, polycyclic aromatic hydrocarbons, etc., are bound to melanin and retained in pigmented tissues for long periods. The physiological significance of the binding is not evident, but it has been suggested that the melanin protects the pigmented cells and adjacent tissues by adsorbing potentially harmful substances, which then are slowly released in nontoxic concentrations. Long-term exposure, on the other hand, may build up high levels of noxious chemicals, stored on the melanin, which ultimately may cause degeneration in the melanin-containing cells, and secondary lesions in surrounding tissues. In the eye, e.g., and in the inner ear, the pigmented cells are located close to the receptor cells, and melanin binding may be an important factor in the development of some ocular and inner ear lesions. In the brain, neuromelanin is present in nerve cells in the extrapyramidal system, and the melanin affinity of certain neurotoxic agents may be involved in the development of parkinsonism, and possibly tardive dyskinesia. In recent years, various carcinogenic compounds have been found to accumulate selectively in the pigment cells of experimental animals, and there are many indications of a connection between the melanin affinity of these agents and the induction of malignant melanoma.

Key words: Autoradiography, Malignant melanoma, Melanin affinity, Parkinsonism

INTRODUCTION

The accumulation of certain chemicals in pigmented tissues, due to melanin affinity, is possibly the most pronounced retention mechanism of the body. For example, chloroquine (Lindquist, 1973) and N,N-bis-acetanilidedimethylamine (Lyttkens et al., 1984) are found in marked concentrations in the melanin of the eye one year after a single i.v. injection in pigmented animals. It is now well established that a large number of substances are bound to melanin, and there are many indications that this is the main factor in the etiology of chronic lesions affecting pigmented tissues (Lindquist, 1973; Larsson, 1979). The selective chemical stress on the pigmented tissues, due to the accumulation and retention of noxious substances on the melanin, may also add to, or even be causally connected with, the early ageing processes that often are seen in melanin-containing cells. The most conspicuous case is perhaps the early graying of hair, which is due to the deterioration of the melanocytes in hair bulbs. Other examples are senile or pre-senile degeneration of melanized structures in the eye and the inner ear, with the development of certain types of cataracts or retinopathy, secondary to degeneration in the retinal pigment epithelium, and hearing loss due to strial atrophy. In the *substantia nigra* of the brain stem, the number of pigmented nerve cells normally decreases at the age of 55–65 years, without impairment of the extrapyramidal system, but an additional loss of neurons, due to the adverse effects of chemicals stored on the neuromelanin, may ultimately cause Parkinson's disease.

In the present paper, the interaction between chemicals and melanin, and its biological implications, is reviewed.

MELANIN AFFINITY

The heterogeneity of substances with melanin affinity is large. Various drugs of quite different categories are represented; psychotropic drugs such as phenothiazines and other neuroleptics and tricyclic antidepressants, drugs for rheumatoid arthritis and malaria, local anaesthetics, aminoglycoside antibiotics and so forth are bound to melanin, as are other kinds of chemicals (herbicides, dyes, alkaloids, metals etc.) (Larsson, 1979). Potts (1962) was the first to demonstrate that phenothiazine drugs accumulate in the uveal tract of pigmented experimental animals, and he could also show that phenothiazines and a number of other polycyclic aromatic compounds are taken up by uveal melanin in vitro (Potts, 1964). Using whole-body autoradiography, Lindquist and Ullberg (1972) found that chloroquine and chlorpromazine are strongly and selectively accumulated and retained in the melanin-bearing tissues

Received April 14, 1993; accepted April 30, 1993.

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of mice, i.e. not only in the uveal tract of the eye, but also in the inner ear (the *stria vascularis* of the cochlea and *planum semilunatum* of the vestibular ampullae) and in the skin—there was no corresponding localization of label in albino animals. During the following years, the melanin affinity in vivo of a great number of substances was documented by autoradiographic techniques (for review, see Lindquist, 1973; Larsson, 1979; Tammela, 1985; Lindquist et al., 1987; Lydén-Sokolowski, 1990). In Figures 1 and 2, the accumulation of chlorpromazine in the melanin-containing tissues of the eye and the inner ear, respectively, is demonstrated.

The binding to melanin varies quite a lot between different substances, and those showing the highest affinity in vitro are mainly organic amines and metal ions (Larsson,

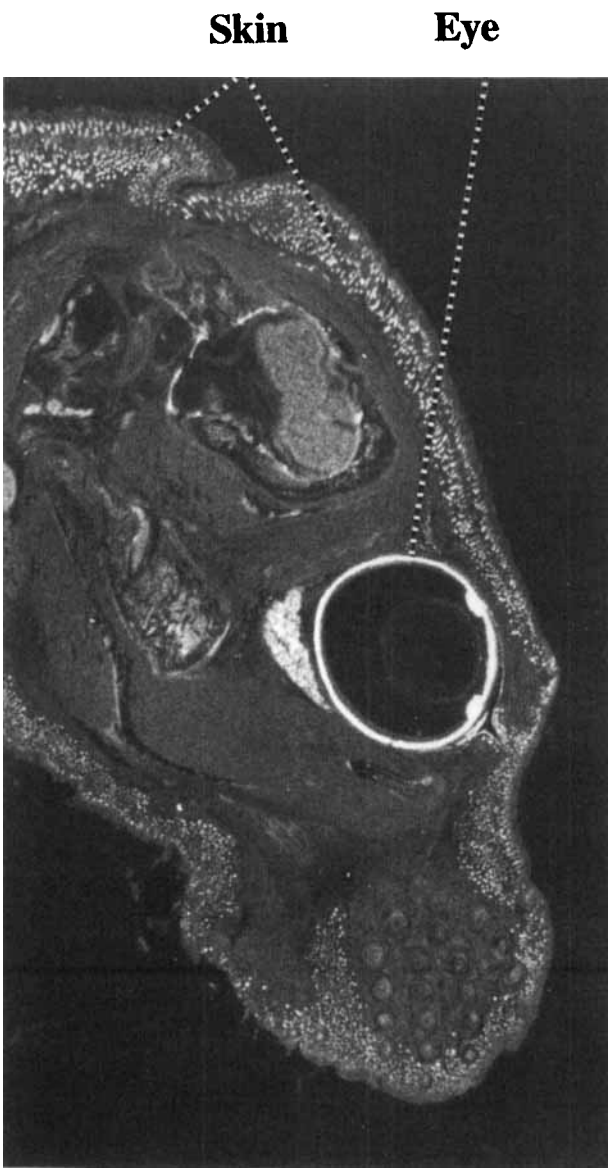

Fig. 1. Autoradiogram of a young hooded rat 1 hour after an intraperitonial injection of ^{35}S-chlorpromazine. There is high accumulation in the melanin of the eye and the skin.

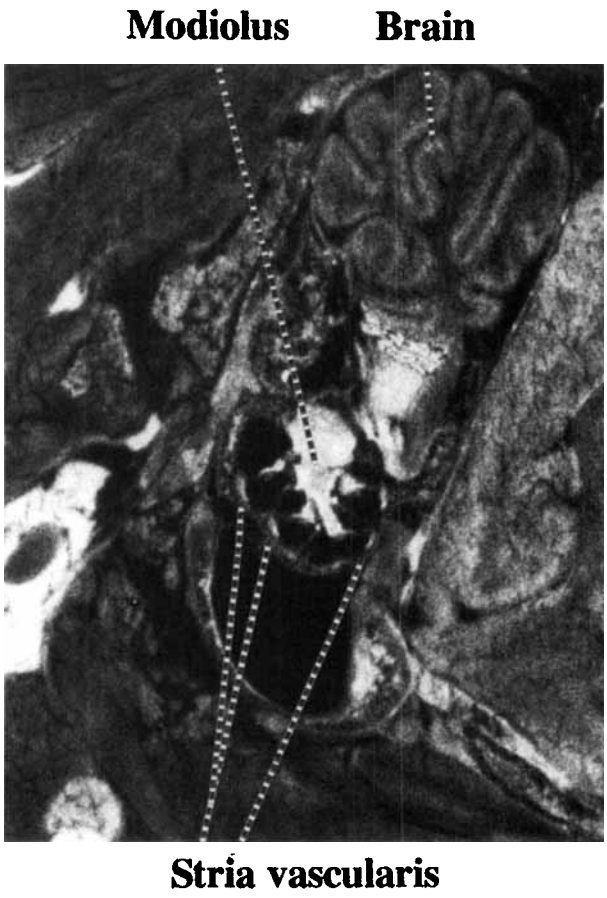

Fig. 2. Detail of an autoradiogram of a young hooded rat 20 min after an intraperitonial injection of ^{35}S-chlorpromazine. Note the selective uptake in the melanin-containing structures of the cochlea in the inner ear (*stria vascularis* and *modiolus*).

1979; Larsson and Tjälve, 1978, 1979). Melanins are polyanions with a relatively high content of negatively charged carboxyl groups and o-semiquinones (Felix et al., 1978; Ito, 1986; Prota, 1992). Substances with cationic properties (e.g., amines and metals) are thus bound to the melanin by ionic interaction (Larsson and Tjälve, 1979). Not only aromatic amines (especially the polycyclic ones) but also aliphatic amines (Tjälve et al., 1981) are bound. The ionic binding is apparently strengthened by other forces, such as van der Waal's attraction at the tight appositions of the aromatic rings of the compounds and the melanin structure. The involvement of charge-transfer interaction has also been indicated for certain electron-donating substances, e.g., chlorpromazine (Potts, 1964; Larsson and Tjälve, 1979), as well as hydrophobic interaction in some cases, which may be rather pronounced (Stepien and Wilczok, 1982; Larsson et al., 1988). The binding is normally complex. Scatchard analyses have shown that more than one binding class is involved for individual substances, including metals, which indicates the presence of cooperating binding mechanisms as well as the influence of steric factors (Larsson and Tjälve, 1979). More recently, Raghavan et al. (1990) reported on studies where confor-

mational analysis and molecular graphics were used to model a representative melanin structure for the estimation of melanin affinity in vitro. They found that the calculated binding energies were inversely proportional to the percentage binding of 13 different chemicals to melanin—the binding data were obtained from Potts (1964). Chlorpromazine, chloroquine, and methylene blue, e.g., which are strongly bound to melanin, showed the lowest binding energies, whereas the reverse was found for phenol and pyridine, which are lacking experimentally provable melanin affinity.

Studies on the melanin affinity in vitro usually give accurate data of the intrinsic interaction, i.e., parameter values of the association constants and the binding capacity of the melanin, but the corresponding outcome of studies in vivo may still be different. This anomaly is mainly due to the interference of some additional factors such as metabolism (biotransformation) and kinetic factors. Aminoglycoside antibiotics, e.g., have a pronounced melanin affinity in vitro, but due to their relatively high molecular weight, combined with positive charges at physiological pH, they are showing a marked extracellular biodistribution (with a few exceptions, mainly due to endocytosis) and do not reach the melanin in vivo (Larsson et al., 1981). Metal ions are also showing high melanin affinity in vitro, but in the living organism rather few metals are accumulated in the pigmented tissues after a single injection (Tjälve et al., 1982; Lydén et al., 1984), apparently due to membrane barriers, or possibly metal binding competition between the melanin and proteins. On the other hand, significant amounts of various metals, e.g., Cu, Mn, Mo, Sr, and Zn, are normally found in pigmented tissues, indicating cumulative accumulation during chronic exposure (Larsson et al., 1991). In this connection it is of interest that the biodistribution of metals may undergo a considerable change after exposure to chelating agents, due to the formation of lipophilic complexes. Lead, for example, is not accumulated in the pigmented tissues of mice after a single injection, but after co-exposure to dithiocarbamates a pronounced melanin binding, both in fetal and adult eyes, is found (Danielsson et al., 1984). Other examples are nickel combined with pyridinethione (Jasim and Tjälve, 1986) and thiram (Borg and Tjälve, 1988). The co-exposure to metals and chelating agents is a potential toxicological problem, since organic compounds with chelating properties are relatively frequent, e.g., in chemical industry, as pesticides, and even as drugs. In general, the lipophilic metal complexes are readily passing the blood-brain barrier, which increases the risk of metal binding to neuromelanin and the induction of central adverse effects.

In addition to the type of melanin affinity of chemicals described so far, i.e., adsorption to preformed melanin, a few melanin-affinic substances (thioureylenes) have been found to be selectively incorporated into nascent melanin (for review, see Larsson, 1991). 2-Thiouracil, which is the most studied substance in this regard, strongly accumulates in the pigmented tissues of fetal eyes, where the rate of melanin synthesis is high, but in the adult eye, where the melanin synthesis is low, only minute amounts of thiouracil are accumulated (Dencker et al., 1979). The uptake in melanin is thus related to the melanin synthesis rather than to the occurrence of preformed melanin—thiouracil is lacking affinity to the preformed melanin (Dencker et al., 1981). A number of thiouracil derivatives (e.g. 5-iodo-2-thiouracil and its 6-alkyl derivatives, thiourea, and methimazol) have also been shown to be incorporated into melanin during its synthesis, and they are all containing a thioureylene group as a structural element, where the sulphur ligand is of crucial importance for the uptake (Olander et al., 1983). The incorporation of thiouracil into melanin is apparently due to covalent binding to dopaquinone, formed during the melanin synthetic pathway, and the thiouracil-dopaquinone adduct is gradually inglobated within the melanin polymer during its formation (Palumbo et al., 1990). The thioureylenes are selectively incorporated into melanotic melanoma (Dencker et al., 1979; Larsson et al., 1982) and much interest has been focused on the clinical potential of using thiouracil derivatives, e.g., 5-iodo-2-thiouracil (Fig. 3), as selective tumour seekers for the diagnosis by radio-scanning or treatment of malignant melanoma (for review, see Larsson, 1991). The risk of adverse effects induced by thioureylenes in normal pigmented tissues with high melanin production, such as the skin or fetal eyes, is so far poorly evaluated. However, it has been reported that thiouracil may cause a specific anomalous gigantism of the melanin granules in the otolith cells of ascidian larvae reared throughout embryogenesis in the presence of the drug, and the morphological changes were also accompanied by a lightening of colour (Whittaker, 1966). It is also known that propylthiouracil and methimazole may cause loss or depigmentation of hair in humans (Goodman and Gilman, 1985), indicating a toxic effect in the hair follicles where the rate of melanin synthesis is normally high.

TISSUE PROTECTION

The physiological meaning of melanin binding is still not fully understood. One possibility might be that the melanin serves as a device for the local regulation of endogenous cations, such as Ca^{2+} (Meyer zum Gottesberge-Orsulakova, 1985). But as far as xenobiotics are concerned, it is more tempting to consider the melanin as a protective chemical filter. The presence of melanin in some very sensitive tissues evidently favours such a hypothesis. The melanin would protect these tissues by keeping potentially harmful substances bound and slowly releasing the agents in low, nontoxic concentrations (especially, transient, high concentration peaks of noxious chemicals are cut by the adsorption to melanin). In the eye, for example, the melanin is located in close proximity to the receptor cells, and the nutrients from the capillary bed of the choroid are filtered through the choroidal melanocytes and the pigment epithelium before reaching the retinal receptors for nourishment. A similar situation exists in the inner ear, because the receptors (the hair cells) are located close to the melanin of the *stria vascularis* in the cochlea and the *planum semilunatum* of the vestibular ampullae. In the brain, neuromelanin is present in nerve cells in the extrapyramidal system, mainly in the *substantia nigra* and *locus coeruleus*, and the neuromelanin may be involved in chemical protection as well.

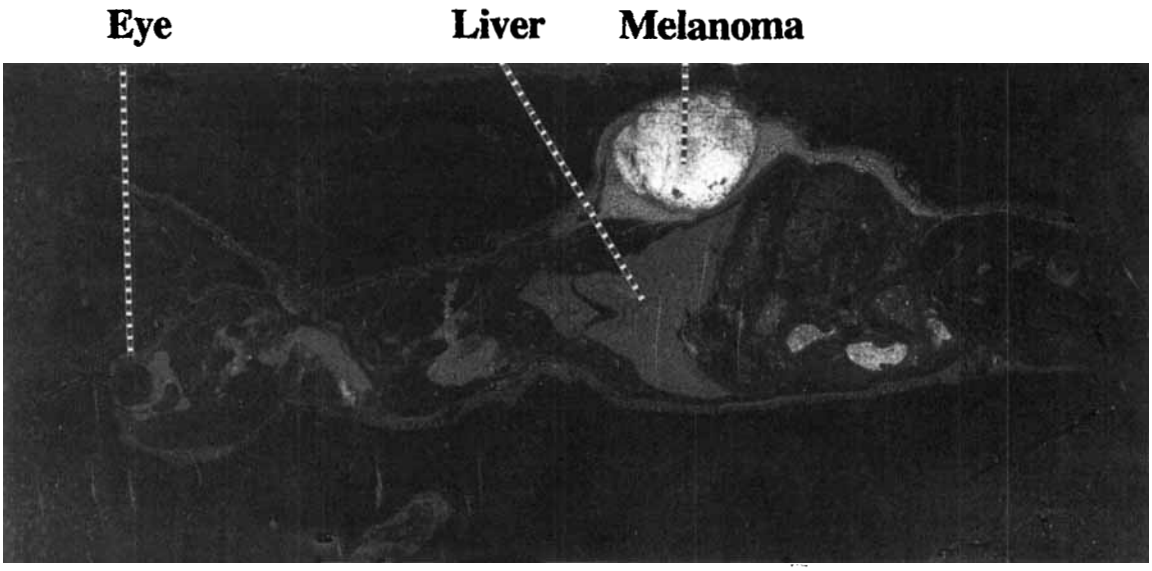

Fig. 3. Whole-body autoradiogram of a mouse, transplanted with Harding-Passey melanoma, 1 day after an intraperitoneal injection of 5-iodo-^{35}S-2-thiouracil. There is high and selective uptake in the tumour.

ADVERSE EFFECTS

Under certain circumstances, however, the possible protection mechanism may be a threat to the pigmented cells. Chronic exposure to certain toxic substances with melanin affinity, for example, ultimately causes adverse effects in the cells. This mechanism seems to be of importance in the development of some chemically induced lesions in the eye, the inner ear, and in the skin, e.g., by phenothiazine derivatives and chloroquine (Lindquist, 1973; Dencker and Lindquist, 1975). The effects are mainly related to high dose/long-term exposure, and a prominent feature of the lesions is that the histologic changes initially are found in the melanin-containing cells, and successively in adjacent tissues, such as receptor cells. The onset of the adverse effects may be delayed, and the entire manifestation of the lesions may occur even years after cessation of the offending substance (Burns, 1966). It is also possible that various toxic substances, which are retained in the pigmented tissues, are causing additive effects. The histopathologic changes (reviewed by Lindquist, 1973) are usually characterized by enlargement of the cells and a marked increase of the number of melanosomes. Gradually, degeneration occurs with the release of melanosomes and other cellular debris, which migrate into surrounding tissues. In the eye, for example, pigment deposits are seen in the retina with atrophy of the photoreceptors. Damage of the melanin-containing cells of the iris may give rise to the release into the aqueous humour of pigment granules which are deposited on the corneal endothelium and the anterior lens surface. In severe cases, they become incorporated and may cause cataracts. The pigment deposits of the eye may be combined with hyperpigmentation of the skin, the so called eye-skin syndrome (Greiner and Berry, 1964). The development of injuries in the pigmented cells of the inner ear follows a similar course, i.e., increased production of melanosomes succeeded by degeneration of the pigmented cells with subsequent secondary impairment of hearing and balance (Dencker et al., 1973).

Treatment with phenothiazines, e.g., chlorpromazine, and other neuroleptics is known to be associated with extrapyramidal disorders such as drug-induced parkinsonism (occasionally with irreversible symptoms) and tardive dyskinesia (Schmidt and Jarcho, 1966; Richardson and Craig, 1982). Although most phenothiazines are bound to melanin (Larsson, 1979), including chlorpromazine, which has been demonstrated to interact with neuromelanin (Lindquist, 1973), it is unclear to what extent the melanin affinity is involved in the extrapyramidal disorders. The syndrome has been claimed to be a hyperdopaminergic condition of the postsynaptic dopamine receptors (Carlsson, 1970), but this hypothesis has been questioned, for example by Christensen et al. (1970), who found structural degeneration in the *striatum* and *substantia nigra* of patients suffering from tardive dyskinesia. More recently, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to cause parkinsonian symptoms with selective destruction of the melanin-containing nerve cells of the *substantia nigra* (for review, see Lindquist et al., 1987; Lydén-Sokolowski, 1990), but with no involvement of the dopamine receptors of the *striatum*, as revealed by positron emission tomography (Hartvig et al., 1986). MPTP is strongly bound to melanin in vitro, including synthetic dopamine melanin (Lydén et al., 1983) and to neuromelanin in vivo (Lindquist et al., 1986). The degree of MPTP-induced neurotoxicity seems to be related to the amount of neuromelanin present in the *substantia nigra*. Man and other primates have much higher sensitivity to MPTP than laboratory animals such as guinea pigs and rats (Chiueh et al., 1983) and mice (Hallman et al., 1984; Heikkila et al., 1984), which almost are lacking neuromelanin (Marsden, 1969; Barden and Le-

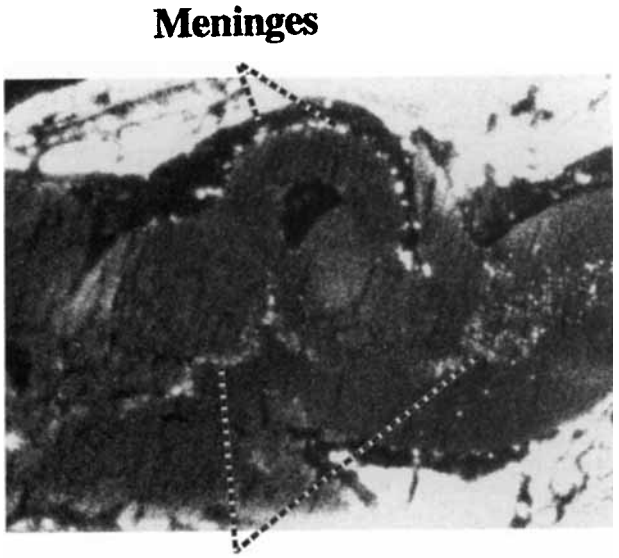

Fig. 4. Detail of an autoradiogram of a frog *(Rana temporaria)* 1 day after an intraperitoneal injection of ^{14}C-labelled paraquat. Accumulation is seen in neuromelanin-containing nerve cells and in melanin-bearing cells in the meninges.

vine, 1983). In the search for possible parkinsonism-inducing candidates, heavy metals such as manganese and compounds structurally related to MPTP have been suggested. Manganese is known to induce an extrapyramidal disorder resembling parkinsonism (Rodier, 1955; Mena et al., 1967), with selective injury of the pigmented nerve cells of *substantia nigra* (Gupta et al., 1980). Like MPTP, manganese is bound to melanin in experimental animals (Lydén et al., 1984). Another candidate is the herbicide paraquat, which is structurally related to MPP^+ (the neurotoxic metabolite of MPTP—cf. below) and shows high melanin affinity (Larsson et al., 1977). Paraquat has been found to induce parkinsonian symptoms in frogs (Barbeau et al., 1985), and by autoradiography (Fig. 4) we have demonstrated selective accumulation of paraquat in the pigmented nerve cells of frogs (Lindquist et al., 1988).

TOXICOLOGICAL MECHANISMS

The mechanism behind the development of lesions in the pigmented cells is probably a combination of selective retention, due to melanin binding, and toxicity, i.e., substances with low toxicity may scarcely induce lesions, in spite of high melanin affinity, while those with a more expressed or specific toxicity may induce the adverse effects. The melanin thus serves as a chemical depot, from which the stored substances are released and slowly enter the cytoplasm (the binding is normally reversible), and the degenerative course of the cell will ultimately be determined by the intrinsic toxicity of the individual substance. Chloroquine, e.g., which induces chorioretinopathy and hearing impairment (cf. above), is known to inhibit various biochemical reactions such as protein synthesis (Roskoski and Jaskunas, 1972) and the digestive activity of lysosomes (Homewood et al., 1972).

The preferential destruction of pigmented nerve cells by MPTP seems to be dependent on local bioactivation in the brain. MPTP is oxidized to the pyridinium cation 1-methyl-4-phenylpyridine (MPP^+) by monoamine oxidase (Chiba et al., 1984)—the biotransformation of MPTP in vivo has also been demonstrated by autoradiographic technique (Lydén-Sokolowski et al., 1988). MPP^+ is bound to neuromelanin (D'Amato et al., 1986), and it has been shown that mainly MPP^+ is responsible for the neurotoxic effects (Langston et al., 1984; Markey et al., 1984). Once stored on the neuromelanin, MPP^+ is continuously released and exerts its toxic effects locally in the pigmented neurons. It has been found that MPP^+ is concentrated in mitochondria by energy dependent mechanisms, and that it inhibits NADH oxidation, leading to ATP depletion and possibly cell death (Ramsay et al., 1986).

It has also been proposed that the binding per se between a substance (mainly electron donors) and the melanin might change the physicochemical properties of the melanin with modification or impairment of its physiological function—there are some indications of a protective role of the melanin in pigmented cells, for example as a sink for free radicals or excited, and potentially harmful, species (for review, see Larsson, 1979). Another possibility, which has been proposed in various connections, might be that melanin participates in redox-cycling reactions with the production of reactive oxygen species such as superoxide anions and peroxides (Swartz et al., 1992).

MELANOMA INDUCTION

The causal association between sun exposure and malignant melanoma is well-documented, but not fully consistent (Koh et al., 1990). In addition to risk factors such as UV exposure and certain individual characteristics, e.g. the number of nevi, ability to tan, skin colour, etc., (MacKie and Aitchison, 1982; Evans et al., 1988), chemical carcinogenesis has been suggested to cause the disease. This hypothesis is supported by a number of studies on the melanoma incidence among workers employed in chemical industries and the like (Pell et al., 1978; Thomas and Decoufle, 1979; Albert et al., 1980; Heldaas et al., 1987; Barthel, 1985; Austin and Reynolds, 1986; Magnani et al., 1987). In autoradiographic studies on the biodistribution of various carcinogenic compounds in experimental animals, we have demonstrated a pronounced and specific accumulation in the melanin-containing cells of aflatoxin B_1 (Larsson et al., 1988), tobacco-specific N-nitrosamines, benzidine, polycyclic hydrocarbons such as dimethylbenz(a)-anthracene and benzo(a)pyrene (Larsson et al., 1989), and some mutagenic food pyrolysis products (Brandt et al., 1983; 1989; Bergman, 1985; Larsson et al., 1989). Most carcinogenic substances are in fact procarcinogens and need metabolic activation to become carcinogenic. It is known that the skin contains inducible enzymes (e.g., aryl hydrocarbon hydroxylase) capable of bioactivating xenobiotics (Pannatier et al., 1978). In a recent study, it was found that human melanocytes are metabolizing benzo(a)pyrene to a number of metabolites, including the proximate carcinogen benzo(a)pyrene-7,8-diol, thus demonstrating the presence of cytochrome p-450 IA1 in the melanocytes (Agarwal et al., 1991). The combination of a specific re-

tention mechanism (reversible melanin binding) and the presence of bioactivating enzymes in pigmented tissues strongly supports the idea of chemical carcinogenesis as an etiological factor behind malignant melanoma, and the results should exhort a more systematic examination of the hypothesis, both epidemiologically and experimentally. There are a few reports on chemical induction of melanoma in experimental animals (e.g., Goerttler et al., 1980; Berkelhammer et al., 1982; Anders et al., 1991), but a problem in this regard is that most studies on chemical carcinogenesis have routinely been performed in albino animals, which are refractory to melanin-related risks. This is also a general problem concerning drug-induced lesions in pigmented tissues.

ACKNOWLEDGMENTS

Acknowledgment is due to Professor Yutaka Mishima for his kind permission to publish this paper which was first presented, in part, at "International Frontiers in Pigment Cell Research," a seminar for cosmetic chemists organized and held under the auspices of Professor Mishima in Tokyo, November 12, 1992, as a support meeting in conjunction with the International Federal of Pigment Cell Societies Board of Councilors' Meeting and the Japanese Society of Pigment Cell Research Annual Meeting.

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