CHAPTER 6

NEUROMELANIN

I. INTRODUCTION

Neuromelanin is a granular, dark brown pigment which is typical of the nigrostrial neurons in the brain stem of humans. Since the early report by d'Azur in 1786, interest in this pigment has continued unabated, especially in view of its possible role in Parkinson's disease. Though the structure of neuromelanin is not known, there are several lines of evidence which suggest that this pigment is chemically different from the melanins formed in epidermal melanocytes. This fact, coupled with the intrinsic importance of the subject, warrants our devotion of this chapter to neuromelanin.

II. OCCURRENCE AND DISTRIBUTION

A comparative survey of the occurrence of neuromelanin in mammals has been reported by Marsden (1961) to involve 49 species representing 11 orders. Though the validity of this study has been questioned by Barden (1981) on the basis of a supposedly incorrect use of the Masson–Fontana staining technique, there seems to be sufficient evidence of the presence of neuromelanin in, besides man, *Hominoidea, Cercopithecoidea, Ceboidea*, and a member of *Lemuroidea*. In general, the amount of neuromelanin in the brain varies in the different animal species, being greatest in man and progressively lower as the relation to man becomes lower, for example, in carnivores and horses. Laboratory animals such as rats, mice, guinea pigs, and rabbits have little or no neuromelanin. Amphibians have been reported to have melanin-containing nerve cells, which may functionally correspond to those in mammals (Kemali and Gioffré, 1985). Several studies of the distribution of neuromelanin in adult human brain, carried out in various laboratories, have shown that the pigment occurs almost exclusively in catecholaminergic neurons (Fig. 6.1), which are involved in conscious perception, movement, emotions, and memory (Bogerts, 1981; Cowen, 1986).

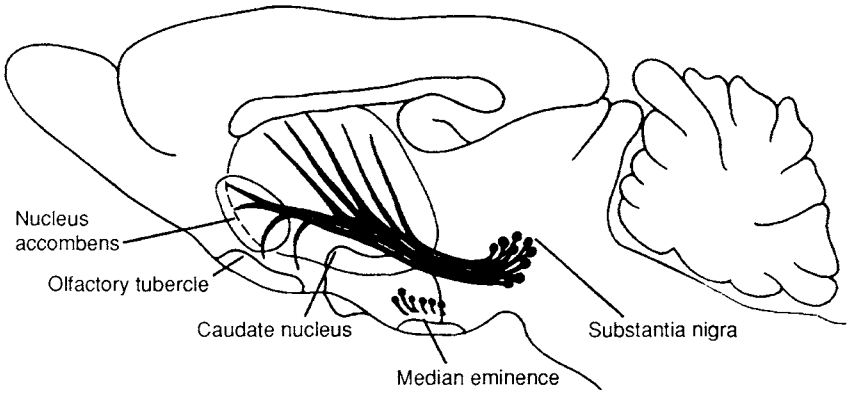


FIG 6.1 Schematic drawing of the brain highlighting dopamine tracts and the neuromelanin-containing *substantia nigra*.

In heavily pigmented neurons, neuromelanin granules are closely packed, dispersed through the cytoplasm, and extend into the axon hillock and initial portions of the axon. Neuromelanin is less abundant in the dendrites and is absent in the neuronal processes remote from the cell bodies, the synaptic nerve endings, and the glia cells. The highest levels of neuromelanin are found in the neurons of the *substantia nigra* and *locus coeruleus*, which are known to contain relatively high concentrations of dopamine and norepinephrine, respectively.

III. ONTOGENESIS AND AGE DECLINE

There is some disagreement as to when neuromelanin makes its appearance in the human brain. The pigment has been described in the *substantia nigra* as early as midterm gestation through 4 years of age, while in the *locus coeruleus* it appears between 5 months gestation and 3 years of age. In the dorsal nucleus of the vagus it appears at 34 weeks gestation (Barden, 1981). During life there is a gradual accumulation of neuromelanin in the dopaminergic neurons with a maximum around 60 years, followed by a decline in the senium (Mann and Yates, 1974). The concentration of neuromelanin around the late eighties is about one-half to two-thirds that of the 60-year level (Fig. 6.2). A similar change in neuromelanin concentration with age has been observed in the brains of rats by Schally and co-workers (Kastin *et al.*, 1979). In general, the heavily pigmented neurons are preferentially lost, suggesting a close link between synthesis or accumulation of neuromelanin and cell degeneration.

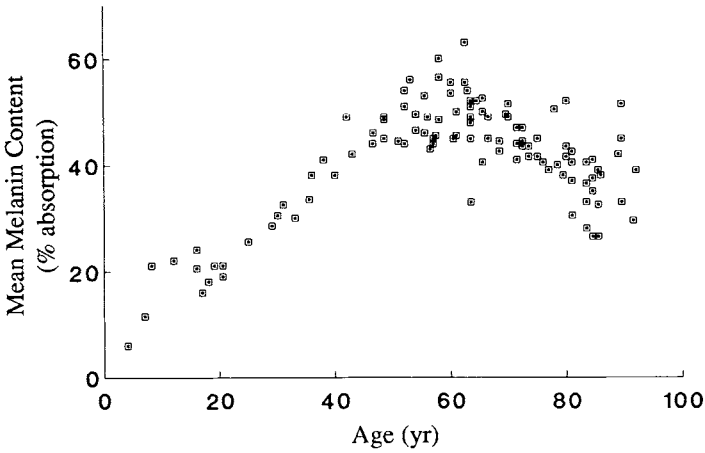


FIG 6.2 The melanin content of nerve cells from the human *substantia nigra* in 110 control subjects between ages 18 months and 91 yr. After Mann and Yates (1974).

The reasons for the decrease in neuromelanin with age are not clear, though it is evidently dependent on a selective loss of dopaminergic neurons, as in Parkinson's disease. Neuromelanin seems to be very stable and is not normally secreted from the cells where it is formed. It may only be released from degenerating nerve cells and phagocytosed by glial cells (Marsden, 1969). Mann and Yates (1977) showed that accumulation of neuromelanin is accompanied first by a reduction in cytoplasmic RNA content and finally by a decrease in nuclear volume in *substantia nigra* neurons. These authors proposed a mechanical disruption of the microanatomy of intracellular membranes by neuromelanin as a mechanism of degeneration of the pigmented dopaminergic cells. Graham (1979) postulated that synthesis of neuromelanin, rather than accumulating, has deleterious consequences for the cells. This view would be in agreement with findings showing that dopamine oxidation occurs more rapidly and the *o*-quinone formed cyclizes more slowly compared to other catecholamines and therefore is highly toxic to the well-being of the dopaminergic cells (Graham, 1978; Cohen, 1983).

IV. MORPHOLOGY AND HISTOCHEMISTRY

Ultrastructural and histochemical studies of neuromelanin granules in the *substantia nigra* and *locus coeruleus* of the human brain indicate similarity to the granules of the brown age pigment lipofuscin with respect to skin and

hair melanosomes. Neuromelanin intraneuronal granules appear to be membrane bound with a lobular configuration and a gross tripartite substructure, composed of vacuolar bodies protruding from a granular or linear matrix on which a very dense particulate material is irregularly deposited. Apparently, lipofuscin granules exhibit a dipartite structure composed of a matrix containing vocuolar bodies but lack the dense particulate component typical of neuromelanin (Barden, 1981).

Both lipofuscin and neuromelanin are positive for acid hydrolase activity and are thought to be lysosomal residual bodies containing end products of oxidative damage of lipids (Barden and Brizzee, 1987). Such a common biochemical feature is supported by the observation that neuromelanin bleached with 10% hydrogen peroxide becomes fluorescent under UV light just like lipofuscin. Moreover, melanin-free lipofuscin from the inferior olive can be melanized if impregnated with ferrous sulfide and incubated with L-dopa. These observations led Barden (1969) to conclude that lipofuscin has the properties of bleached neuromelanin and that neuromelanin is melanized lipofuscin. Histochemical experiments, reviewed by Barden (1981), showed that the tinctorial characteristics of neuromelanin reflect fairly well the tripartite structure of the pigment granule as apparent from electron microscopy. Thus, neuromelanin is histochemically divisible into three components: (1) the melanin component, detectable by ferrous and cupric ion uptake reactions, a green staining with Nile blue sulfate, and the bleaching reaction; (2) the lipoproteinlike lipofuscin component, corresponding to the matrix, characterized by several staining reactions all duplicated in inferior olive lipofuscin; and (3) the unbound lipid lipofuscin component, corresponding to the vacuolar component, characterized by staining with naphthol sudans, Sudan III and IV and Oil red 0.

V. BIOSYNTHESIS

A. *Precursors*

The apparent association of neuromelanin with the catecholamine tracts of the brain is generally regarded as indirect proof of the involvement of dopamine and related metabolites in the biosynthesis of the pigment. Under normal circumstances, this oxidative pathway appears to be only a minor part of the overall metabolism of catecholamines, which involves mainly oxidative deamination by monoamine oxidase (MAO) and *O*-methylation by catechol *O*-methyl transferase (COMT).

A major question that had to be resolved by the first investigators was which of the two main catecholamines in the brain, namely dopamine and norepi-

TABLE 6.1
DOPAMINE AND 5-*S*-CYSTEINYLDOPAMINE IN HUMAN BRAIN[a]

	Dopamine[b]		5-*S*-cysteinyldopamine[b]	
	Mean	(Range)	Mean	(Range)
Caudate nucleus	1.2	(0.75–1.6)	0.05	(0.03–0.09)
Putamen	1.3	(0.99–1.6)	0.06	(0.02–0.09)
Globus pallidus	0.74	(0.58–0.95)	0.01	(0.008–0.016)
Substantia nigra	0.31	(0.11–0.46)	0.02	(0.007–0.024)
Cerebellum	0.06	(0.02–0.13)	0	
Occipital cortex	0.13	(0.08–0.20)	0	

[a]From Rosengren *et al.* (1985).
[b]μg/g fresh weight

nephrine, is the actual precursor of neuromelanin. Some useful information was obtained by Das *et al.* (1978), who compared the absorption spectra of various melanin preparations before and after solubilization with sodium borohydride. They found that the spectroscopic characteristics of neuromelanin from human *substantia nigra* matched fairly well with those of synthetic dopamine melanin, but not with those of dopamelanin or other catecholamine melanins. Likewise, Bridelli *et al.* (1982) reported that the IR spectrum of neuromelanin has features similar to those of synthetic dopamine melanin.

More recently, Rosengren *et al.* (1985) have reported the occurrence of a new dopamine metabolite, 5-cystein-*S*-yldopamine (5-cysdopamine), in several dopaminergic regions of the brain (Table 6.1). The identification was secured by direct comparison with a synthetic sample prepared by addition of cysteine to dopaminequinone, as outlined in Fig. 6.3.

This finding, coupled with the high levels of sulfur in neuromelanin (Barden and Martin, 1972), would support the hypothesis that dopamine is oxidized to dopaminequinone which is in part converted to cysteinyldopamine, acting as an additional pigment precursor (see Section VI).

B. *Oxidative Systems*

Very little is currently known regarding the nature of the enzymatic system(s) involved in the oxidative process of neuromelanin formation. The presence of tyrosinase in melanin-producing tissues led Marsden (1965) to invoke a role of this enzyme in neuromelanogenesis in human *substantia nigra*. Support for this view was provided by *in vitro* experiments showing that cells of the

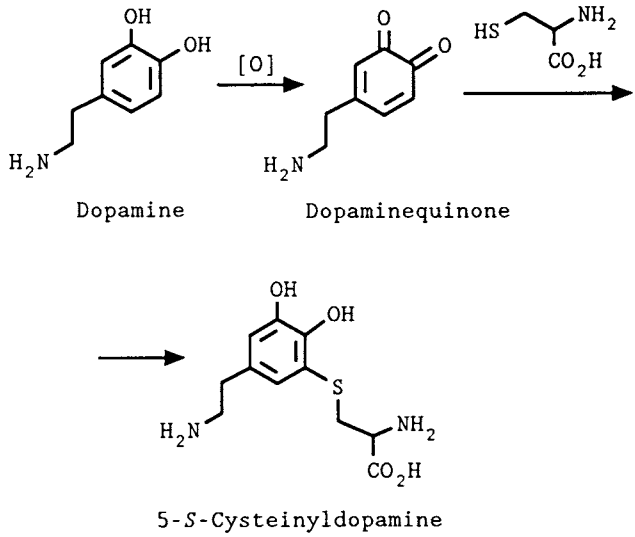


FIG 6.3 Formation of cysteinyldopamine by addition of cysteine to enzymatically generated dopaminequinone.

substantia nigra of adult monkey and cat are blackened on incubation with L-dopa. Histochemical studies by various other workers, however, were unable to confirm tyrosinase in the brain, and all biochemical estimations of this enzyme were unequivocally negative (Barden, 1981). The normal pigmentation of the *substantia nigra* in human albinos (Foley and Baxter, 1958), who lack all peripheral tyrosinase, is also consistent with tyrosinase not being involved in neuromelanin formation.

Another enzyme which has been implicated is peroxidase, which *in vitro* rapidly converts dopamine into a black melanin pigment (Okun *et al.*, 1972). Moreover, in one isolated case of unilateral Parkinsonism a 20-fold decrease in peroxidase activity was observed in affected basal ganglia. Histochemical and biochemical detection of peroxidase in cells of the dopaminergic system have also been reported (Ambani *et al.*, 1975); nonetheless, its role in neuromelanin synthesis has been questioned by Rodgers and Curzon (1975) and, more recently, by Rabey and Hefti (1990), who found that addition of hydrogen peroxide to *substantia nigra* homogenates produces modest increases in melanin formation. Likewise, addition of catalase, which destroys endogenous hydrogen peroxide, failed to significantly inhibit melanin formation below values measured in control homogenates.

The role of MAO appears more relevant to neuromelanogenesis. At a time when tyrosinase was ruled out as the enzyme responsible for pigment produc-

tion in the brain, Cote (cited in Marsden, 1969) found an apparently similar melanin-forming enzyme activity in the *substantia nigra* of *Rhesus* monkey and identified it as MAO by inhibition experiments. This view received alternate consideration until recently, when Rabey and Hefti (1990) reported new evidence that MAO, coupled with nonenzymatic oxidative processes, is mainly responsible for neuromelanin formation. It is clear, however, that the definitive assessment of these results awaits further experimental support. In this context, more attention should perhaps be placed on autooxidative processes catalyzed by redox metal ions, especially iron, which is known to accumulate in the *substantia nigra* (Youdim *et al.*, 1989).

VI. ISOLATION AND STRUCTURAL INVESTIGATIONS

At present little is definitely known about the structure of neuromelanin: Even the elemental composition is uncertain. This is largely due to the relative inaccessibility of the *substantia nigra*, the exceedingly low amounts of neuromelanin present, and the difficulties connected with liberating the pigment granules from the surrounding lipid matrix in which they are deeply embedded. In early studies (e.g., Van Woert *et al.*, 1967; Das *et al.*, 1978) a complex purification procedure was used involving prolonged proteolytic digestion with trypsin or pronase, treatment with lysing solutions, extensive extractions with organic solvents, and in some cases drastic acid hydrolysis with 6 *M* HCl. Spectroscopic studies of the pigment preparations thus obtained did not provide evidence for any distinguishing structural feature except a vague similarity of the absorption spectrum to that of dopamine melanin (Fig. 6.4).

Recently, an improved procedure for isolating human *substantia nigra* neuromelanin has been developed by Rorsman, Rosengren, and co-workers (Carstam *et al.*, 1991). This involves extraction of lipids and proteins with 2% sodium cholate in 30% ethanol followed by 2% SDS in 10% glycerol. Reductive hydrolysis of the pigment thus obtained with hydroiodic acid and real phosphorous yielded 4-amino-3-hydroxyphenylethylamine in large quantities along with trace amounts of 4-amino-3-hydroxypenylalanine (AHP), indicating that 5-cysdopamine and 5-cysdopa, to a lesser extent, both concur to form the polymer. Dopamine and small quantities of dopa were also obtained by hydrolysis, whereas permanganate oxidation afforded some PTCA. These data would convey an image of neuromelanin as a "mixed-type" melanin derived from dopamine, in which pheomelanic- and eumelanic-type components coexist, probably as a result of an exhaustion of the GSH–cysteine defense system of the *substantia nigra* neurons.

Ongoing work in other laboratories, however, does not seem to provide confirming evidence for this structural view. Wakamatsu *et al.* (1991) failed to

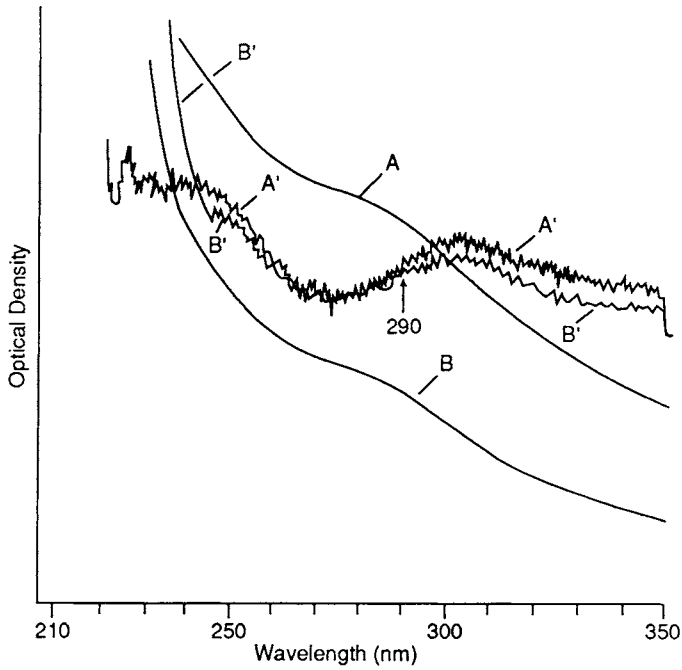


FIG 6.4 Absorption spectra of dopamine melanin (A) and *substantia nigra* melanin (B) solubilized in sodium borohydride. A' and B' represent the first derivative spectra of A and B respectively.

detect cysdopamine-derived units in human *substantia nigra* melanin. On the other hand, Zecca *et al.* (1992) reported analytical and spectroscopic data suggesting that 5-cysdopa or 5-cysdopamine linked to a palmityl residue are the most abundant constituents of human neuromelanin. It is therefore clear that the current picture of neuromelanin structure is still very uncertain and susceptible to profound modifications in the near future.

VII. MODEL STUDIES

A. Dopamine Melanin

Most of what is currently known about the structure of dopamine melanin is due to the intensive studies of Swan (1974) on the pigment obtained by autooxidation of dopamine. This was found to be soluble in dilute akali, and when the resulting solution was shaken with benzoyl chloride a brownish

product was obtained which was partly soluble in chloroform. By using ^{14}COCl-benzoyl chloride, the product was shown to contain about one benzoyl group per 8-carbon unit in the polymer. Moreover, the presence of two carbonyl stretching bands in the IR spectrum (1746 cm^{-1}, strong, and 1646 cm^{-1}, weak) indicated that benzoylation had occurred mainly at the phenolic hydroxyl groups and, to a lesser extent, at amino groups.

More insight into the structure of dopamine melanin was obtained by oxidation of samples of dopamine, specifically deuterated at the α and β positions of the side chain, and at the 2, 5, and 6 positions of the benzene ring (Binns *et al.*, 1970b). In all cases, the ratio of deuterium enrichment in the pigment to that in the precursor was higher than that found for the same position of labeling in dopamelanin, as if any of the carbon atoms in the dopamine molecule was involved in other types of polymeric linkages. In other words, a considerable number of the units in dopamine melanin must be held together by C-N or C-O linkages, in contrast to dopamelanin, where only C-C linkages seem to be involved.

Analysis of the incorporation values led Swan to suggest that dopamine melanin was a copolymer containing a high proportion of uncyclized dopamine units and DHI units, along with some carboxylated pyrrolic units arising by peroxidative cleavage of the benzene ring of what were originally 1,7-linked indole units. The percentage composition of the proposed units and the positions involved in the polymeric linkages are indicated in Fig. 6.5.

Swan's conclusions are attractive in connecting a number of different data. However, they are based entirely upon the assumption that all measured radioactivity originated from a high-molecular-weight polymer rather than a mixture of oligomers of low molecular weight, as suggested by the pigment solubility and easy formation of derivatives. Yet, in spite of this and other doubts, a remarkable difference between dopamine melanin and dopamelanin is the presence in the former of a high proportion of uncyclized side chains, as indicated also by ^{13}C NMR spectroscopy (Peter and Förster, 1989). The reason for such a difference is probably due to the minor tendency of dopaminequinone with respect to dopaquinone to undergo intramolecular cyclization (Young and Babbitt, 1983; Jimenez *et al.*, 1984). This implies that at high substrate concentrations, like those used to obtain dopamine melanin, there may be competition for reaction at the 6 position of dopaminequinone between intramolecular cyclization and intermolecular addition of the amino group of another molecule of dopamine.

There would also be the possibility that dopaminequinone could react with the hydroxyl ion to give 6-hydroxydopamine. In support of this, Senoh and Witkop (1959a,b) reported that dopamine can be converted into 6-hydroxydopamine by the rat, as well as *in vitro*, and that the latter readily undergoes oxidation to give the *p*-quinonoid aminochrome (Fig. 6.6). This,

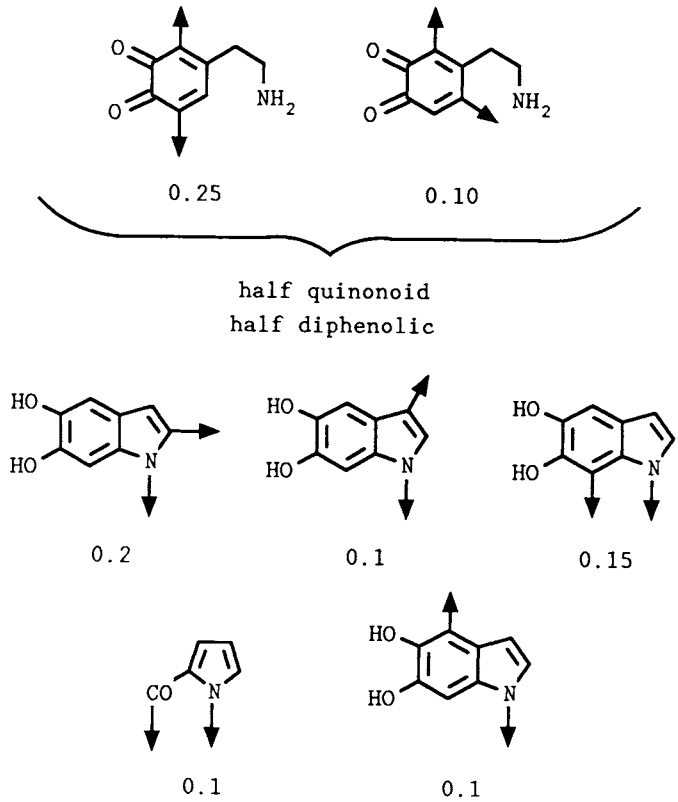


FIG 6.5 Percentage composition of the units present in dopamine melanin according to Swan (1976). Arrows indicate the position of attachment of the units in the pigment polymer.

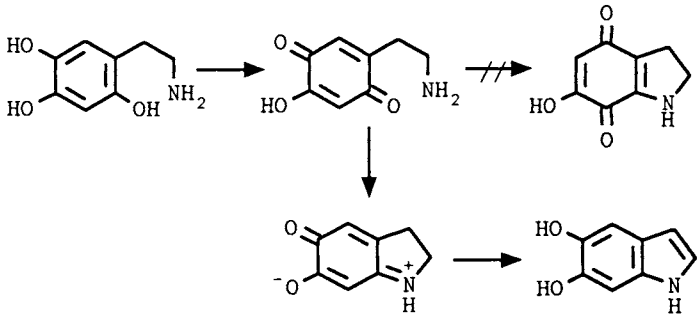
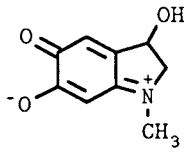


FIG 6.6 Oxidative cyclization of 6-hydroxydopamine.

however, was not isolated and its formation seems unlikely in light of a previous work by Harley-Mason (1953), later confirmed by Swan (1976), showing that oxidative cyclization of 6-hydroxydopamine proceeds as expected to give dopachrome and then DHI.

B. Adrenalin Black

The early stages of adrenalin oxidation have long been known and involve formation of adrenochrome (6.**1**) and its rearrangement to adrenolutin (6.**2**) (Heacock, 1965). The subsequent fate of these intermediates was until recently very little understood. In 1945 Cohen suggested that oxidation of adrenochrome leads to a dark red pigment, "oxoadrenochrome," which was tentatively identified as the *o*-quinone of adrenolutin (6.**3**). This structure was



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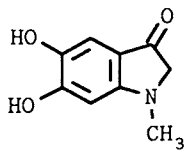

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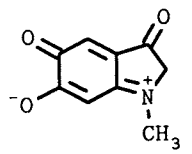

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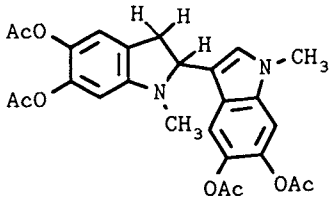
later questioned by Harley-Mason (1950), who failed to obtain oxoadrenochrome by oxidation of adrenolutin: The only recognizable product was a greenish-black pigment of indigoid nature. Moreover, he found that when adrenochrome was treated with acids or was left in aqueous solution under nitrogen for 24 hr, it changed nonoxidatively into a dark pigment termed "adrenalin black." This was an amorphous material insoluble in organic solvents, except pyridine, but soluble in sodium hydroxide solution, from which it could be reprecipitated with acids.

Based upon chemical and spectrophotometric experiments and partly on theoretical grounds, Bu'Lock (1961) suggested that formation of adrenalin black proceeded via the initial acid-catalyzed dehydration of adrenochrome to give 1-methyl-5,6-indolequinone, which subsequently polymerized through the 3 and 4 positions of the indole ring. In contrast with this view, a reexamination of the structure of adrenalin black (Corradini *et al.*, 1988) showed that the pigment consists of a complex mixture of oligomers of 5,6-dihydroxy-1-methylindole, some of which (present in somewhat greater amounts) have been isolated and characterized as the dimers 6.**4** and 6.**5** and the trimer 6.**6**. The structure of these products clearly indicates that pigment formation proceeds by a mechanism which involves the preliminary conversion of adrenochrome to 5,6-dihydroxy-1-methylindole, followed by acid-catalyzed enamine–imine type dimerization of the indole through the 2 and 3 positions.



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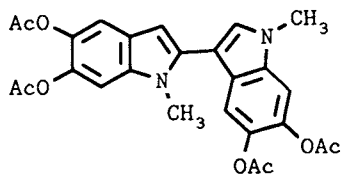

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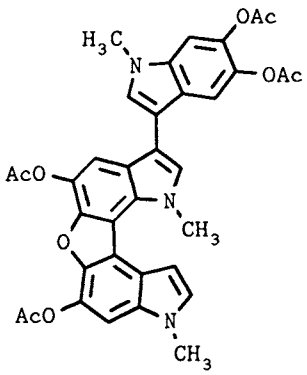

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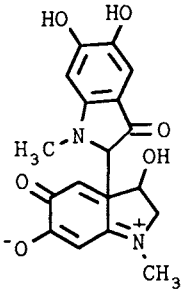
In an extension of this study (d'Ischia *et al.*, 1988) it was found that at physiological pH values, under anaerobic conditions, the major product of adrenochrome rearrangement is not adrenolutin, as generally believed, but a yellow dimeric product (6.**7**) consisting of an adrenolutin moiety covalently linked to the angular 9 position of adrenochrome. When air was allowed into the solution, the reaction took a more complex course, due to the formation, besides the dimer, of two additional products arising from autooxidation of adrenolutin. These were identified as 5,6-dihydroxy-1-methylisatin (6.**8**) and the corresponding 4,4'-dimer (6.**9**).

The product distribution of adrenochrome rearrangement was markedly dependent on the initial concentration of the aminochrome, the nature of the buffer and, notably the pH of the medium (Palumbo *et al.*, 1989). In particular, at pH higher than 8 the reaction was mainly directed toward the formation of adrenolutin, whereas at pH around or below neutrality both the dimer and 5,6-dihydroxy-1-methylindole prevailed. Transition metal ions of common occurrence in biological systems also affect adrenochrome decomposition, to an extent and with modalities which depend on the nature of the metal. Thus, redox-inactive or oxidizing cations, like Zn^{2+} and Cu^{2+}, markedly



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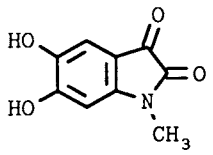

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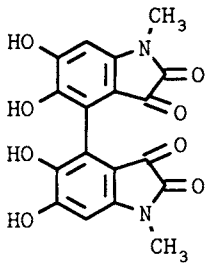

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hasten the rate of rearrangement, inducing the formation of adrenolutin. Mild reductants like Fe^{2+} ions, on the other hand, cause adrenochrome to follow an entirely different decomposition pathway, involving reduction to leucoadrenochrome and its subsequent dehydration to 5,6-dihydroxy-1-methylindole. The results of these studies point to a profound difference in the oxidative pathways of dopamine and adrenalin, which is evidently due to the presence of the β-hydroxyl group affecting the reactivity of the resulting indole metabolites.

VIII. FUNCTIONAL SIGNIFICANCE OF NEUROMELANIN

Although the role of neuromelanin is not known, studies of the physicochemical properties of the pigment have given rise to several hypotheses that may or not be mutually related (Lacy, 1981; Youdim *et al.*, 1989; Rabey and Hefti, 1990). One view is that neuromelanin could function as a device for the inactivation of harmfully excited molecules into innocuous vibrational energy (McGinness and Proctor, 1973). Other authors have postulated that neuromelanin could exert a protective role by acting as a sink for free radicals (Commoner *et al.*, 1954) or as a redox buffer against reducing or oxidizing toxic conditions (Gan *et al.*, 1976, 1977). Another property of neuromelanin, which is typical of eumelanins (see Chapter 4), is the ability to bind a number of foreign substances and to retain them for a very long time (Ings, 1984). This has led Lindquist *et al.* (1987) to suggest that neuromelanin may protect the

surrounding cells by keeping potentially harmful substances bound and thereafter slowly releasing them in low, nontoxic concentrations.

An interesting theory, proposed by Forrest (1974), envisages neuromelanin as serving a biocybernetic function, whereby its lack or depletion causes the *substantia nigra* to revert from its neuroendocrine role to its original motoric role. Moreover, it would inhibit the bioelectric activity of the neuron, producing a decrease in the cell's discharge frequency by controlling the electron flow from the cell cytoplasm into the original axon cylinder. Which of these theories has the greatest physiological relevance is at present difficult to assess, as too little is known on the origin and metabolism of neuromelanin.

IX. NEUROMELANIN AND PARKINSON'S DISEASE

Though degeneration of the *substantia nigra* in the *striatum* is undoubtedly associated with Parkinson's disease, as carefully documented by Hirsch *et al.* (1988), there is a question as to whether or not neuromelanin may have a part to play in the induction of parkinsonism and as to whether it may be either a cause or a consequence of the degradation of the dopaminergic neurons. Marsden (1983) argues that neuromelanin is not directly related to the pathogenesis of parkinsonism and that the Lewy body, an inclusion in cells of the dopaminergic system involved in the disease, may hold the key.

An alternate view stems from the aforementioned ability of neuromelanin to bind foreign substances, some of which may be a threat to the pigment cell, as suggested by certain forms of chemically induced parkinsonism (Ings, 1984). Typically, chronic exposure to manganese dust in miners is known to affect the nervous system with symptoms similar to those in Parkinson's disease (Lyden *et al.*, 1984). The damage seems to especially hit the melanin-containing cells of the *substantia nigra*, presumably by a mechanism involving metal-catalyzed autooxidation of dopamine with formation of cytotoxic quinones (Donaldsson *et al.*, 1982; Graham, 1984). Another neurotoxin with a high affinity for neuromelanin is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a meperidinelike narcotic drug which readily penetrates the blood–brain barrier giving rise to severe parkinsonian symptoms (Lindquist *et al.*, 1987; D'Amato *et al.*, 1986; Kopin *et al.*, 1986). Once in the brain, MPTP is converted in a two-step process (Fig. 6.7) to the 1-methyl-4-phenylpyridinium ion (MPP^+) which is believed to be the actual substance responsible for the neuronal destruction (Castagnoli *et al.*, 1985; Langstron *et al.*, 1987).

Poirer *et al.* (1985) have postulated that a radical intermediate in the initial oxidation of MPTP may potentiate the autooxidation of dopamine to dopaminequinone. However, a pulse radiolysis study of the decay of one-electron oxidized MPTP in the presence and in the absence of dopamine gave a

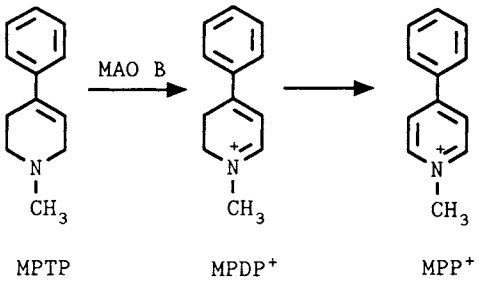

FIG 6.7 Bioactivation of MPTP.

rate-constant limit of $< 2 \times 10^7 M^{-1}s^{-1}$ for the reaction, suggesting that the proposed pathway is unlikely to be of importance in the development of Parkinson's disease (Chacon *et al.*, 1987; Land, 1988).

The degree of MPTP neurotoxicity seems to be related to the amount of neuromelanin present in the brain. Primates, including man, have much higher sensitivity to MPTP than laboratory animals, for example, mice (Heikkila *et al.*, 1984). Many other compounds, like MPTP and manganese, have melanin affinity and may cause lesions in pigmented neurons secondary to their accumulation on the neuromelanin granules. Among these, of particular interest is the pesticide paraquat, which is structurally similar to MPP^+. In a recent epidemiological investigation, the highest prevalence of Parkinson's disease was found in rural agricultural areas of high pesticide use (Barbeau *et al.*, 1986).

Other substances capable of inducing selective degeneration of catecholamine neurons in animals are 6-hydroxydopamine and 6-aminodopamine (Graham *et al.*, 1978; Morrison and Cohen, 1983). The selective toxicity of these molecules has been ascribed to the affinity for the uptake mechanisms of catecholaminergic neurons, coupled with their marked tendency to undergo autooxidation with formation of free radicals, particularly the hydroxy radical (Cohen, 1983). The superoxide ion radicals have also been implicated in the mechanism of cytotoxicity, but a recent pulse radiolysis study does not seem to support this notion (Kalyanaraman *et al.*, 1988).

It is well known that oxygen toxicity is dependent on normal functioning of antioxidant systems such as GSH, GSH peroxidase, SOD, and ascorbate, present in relatively high concentrations in the basal ganglia. A deficiency in any of these would inevitably make the dopamine neurons much more sensitive to chemical insults. Thus, ultimately, the balance between production and disposition of free radicals may be an important factor in the pathogenesis of parkinsonism.