The roles of neuromelanin, binding of metal ions, and oxidative cytotoxicity in the pathogenesis of Parkinson’s disease: a hypothesis

W. S. Enochs¹,², T. Sarna³, L. Zecca⁴, P. A. Riley⁵, and H. M. Swartz¹,⁶

¹University of Illinois College of Medicine, Urbana, IL, and
²Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, U.S.A.
³Jagellonian University, Krakow, Poland
⁴Istituto Technologie Biomediche Avanzate, CNR, Milano, Italy
⁵University of London, London, United Kingdom
⁶Dartmouth Medical School, Hanover, NH, U.S.A

Accepted December 13, 1993

Summary. A characteristic feature of both Parkinson’s disease (idiopathic paralysis agitans) and normal aging is loss of pigmented neurons in the substantia nigra. This has been found to correlate with the accumulation of neuromelanin and with oxidative stress in this brain region, but a clear association between these factors has not been established. Based on our recent demonstration that neuromelanin is a true melanin containing bound metal ions in situ, we present a general model for its accumulation in vivo and the hypotheses (1) that it has a cytoprotective function in the sequestration of redox-active metal ions under normal conditions but (2) that it has a cytotoxic role in the pathogenesis of Parkinson’s disease. Thus, neuromelanin accumulates normally through the autooxidation of catecholamines and serves tightly to bind redox-active metal ions, processes which would accelerate under conditions of intracellular or extracellular oxidative stress. Based on the known properties of melanin, however, neuromelanin also has the potential for exacerbating oxidative stress, e.g., by generating H₂O₂ when it is intact or by releasing redox-active metal ions if it loses its integrity; these reactions also would modulate the reactivity of the neuromelanin. By overwhelming intracellular antioxidative defense mechanisms, such a positive-feedback cycle could turn a condition of chronic or repeated oxidative stress in vulnerable neurons into an acute crisis, leading to cellular death. If the cumulative stress in duration and/or degree is severe enough, neuronal depletion could be sufficient to cause Parkinson’s disease during life. One possible trigger for this cascade is suggested by the increased nigral iron contents in postmortem parkinsonian brains and the correlation of this disease with urban living where exposure to heavy metal ions is high:
the saturation of neuromelanin with redox-active metal ions. Parkinson’s
disease therefore may be a form of accelerated aging in the substantia nigra
associated with environmental toxins in which neuromelanin has a central,
active role.

**Key words:** Neuromelanin, melanins, metal ions, free radicals, oxygen
toxicity, substantia nigra, aging, Parkinson’s disease, idiopathic paralysis
agitans.

**Introduction**

Parkinson’s disease (PD, idiopathic paralysis agitans) is a neurological dis-
order of the extrapyramidal system characterized clinically by akinesia,
rigidity, and tremor (Stern, 1990). Its most consistent pathological finding is
extensive degeneration of the naturally pigmented dopaminergic neurons
projecting from the pars compacta of the substantia nigra to the striatum
(caudate nucleus and putamen) (Greenfield and Bosanquet, 1953; Pakken-
berg and Brody, 1965), which causes a functional deficiency of dopamine in
the latter area (Hornykiewicz, 1966; Bernheimer et al., 1973). In both PD
and normal aging, the most highly pigmented neurons in this brain region
are most susceptible to death (Pakkenberg and Brody, 1965; Graham, 1979;
Mann and Yates, 1979, 1983; Hirsch et al., 1988). Although a parkinsonian
syndrome similar to PD has been shown to result from acute exposure to 1-
methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983;
Singer et al., 1987; Adams and Ogunze, 1991b), the etiologic mechanism
of idiopathic PD remains unknown. Proposed causes of the latter have
included viral infection (Elizan and Casals, 1983; Mattock et al., 1988),
artherosclerosis (Issidorides, 1971; Bernheimer et al., 1973), heredity (Poirer
et al., 1991; Tanner, 1992), environmental and endogenous toxins (Langston
et al., 1987; Fahn, 1989; Goldsmith et al., 1990; Poirier et al., 1991;
Furtado and Mazurek, 1991; Tanner, 1989, 1992), and mechanical disruption
of neurons by accumulating neuromelanin (Mann and Yates, 1977, 1983).

Since the discovery of parkinsonism induced in primates by exposure to
MPTP, research into the etiology of idiopathic PD in humans has intensified,
and strong circumstantial evidence has accrued suggesting that neuronal loss
in the substantia nigra may result from lethal oxidative stress [for reviews,
Fahn (1989), Halliwell (1989), Youdim et al. (1989), Martilla and Rinne
(1989), Olanow (1990), Götz et al. (1990), Ben-Shachar and Youdim (1990),
Adams and Ogunze (1991a,b), Olanow (1992), Jenner (1992), Jenner et al.
(1992), Hirsch (1992), Dexter et al. (1992), Ben-Shachar et al. (1992)]. This
largely is based on: the production of hydrogen peroxide occurring with
the normal and abnormal metabolism of dopamine (Cohen, 1983, 1986;
Graham, 1984); increased amounts of redox-active metal ions, principally
iron, observed in diseased substantia nigra and the known ability of such
ions in vitro to reduce hydrogen peroxide to the potentially highly toxic
hydroxyl radical (Halliwell, 1989; Adams and Ogunze, 1991b; Dexter et al.,
The role of neuromelanin in Parkinson's disease

1992; Riederer et al., 1992; Ben-Shachar et al., 1992); evidence for a disease-related reduction in antioxidative capacity in this brain region (Jenner, 1992; Jenner et al., 1992); and the presence of increased products of lipid peroxidation (Jenner, 1992; Jenner et al., 1992). While a role for neuromelanin in this process occasionally has been suggested (Lindquist et al., 1987; Youdim et al., 1989; Ben-Schachar and Youdim, 1990; Ben-Shachar et al., 1992; Hirsch, 1992; Jellinger et al., 1992), however, such a role has not been clearly defined nor has an attempt been made to relate the preceding observations to the accumulation of neuromelanin.

By combining previous and current work by others with recent results from our laboratories, we present a comprehensive hypothesis for the pathogenesis of PD which centers on an active role for neuromelanin. This is contrary to prior suggestions that it has a passive role, such as the mechanical disruption of neurons (Mann and Yates, 1977, 1983) or the binding and concentrating of environmental toxins similar to MPTP (Lindquist et al., 1987; D'Amato et al., 1987; Fahn, 1989). Our proposal provides a general model for the accumulation of neuromelanin in catecholaminergic neurons and suggests a cytoprotective function for it under normal conditions. However, it also offers a plausible mechanism for the depletion of pigmented neurons during normal aging and its acceleration in PD, postulating a specific etiologic trigger for the latter that is consistent with the cytoprotective function.

Nature of neuromelanin

Neuromelanin is a poorly-defined pigment [for reviews, see Marsden (1969), Van Woert and Ambani (1974), Barden (1975, 1981), Barden and Brizzone (1987)] that accumulates normally with age in the central catecholaminergic neurons of most species (Bazelon and Fenichel, 1967; Mann and Yates, 1974; Bogerts, 1981). It is most abundant in the substantia nigra and locus coeruleus (which are dopaminergic and noradrenergic nuclei, respectively, in the mesencephalon) (Bazelon and Fenichel, 1967; Bogerts, 1981) and increases in amount between species according to their phylogenetic proximity to man (Marsden, 1961). Histological studies have demonstrated that neuromelanin occurs as a set of membrane-limited granules within the neuronal perikaryon (Moses et al., 1966; Hirogawa, 1968), while extensive histochemical analyses provide strong evidence that: these granules are inactivated lysosomes; the pigment occurs in association with lipofuscin; and it has a high content of sulfur [for reviews, see Barden (1975, 1981), Barden and Brizzone (1987)]. Spectroscopic and degradative studies further indicate that a major precursor of neuromelanin is the catecholamine neurotransmitter specific to the pigmented neuron (e.g. dopamine in the substantia nigra and norepinephrine in the locus coeruleus) (Van Woert et al., 1967; Maeda and Wegmann, 1969; Nordgren et al., 1971; Das et al., 1978; Carstam et al., 1991; Zecca et al., 1992).

Based on results with the usual histochemical and physicochemical methods for detecting melamins, neuromelanin has been classified as a
melanin (Lillic and Yamada, 1960; Marsden, 1969; Van Woert and Ambani, 1974; Barden, 1975, 1981; Barden and Brizzi, 1987); however, these methods often give ambiguous results (Sarna and Swartz, 1978). By comparison, electron paramagnetic resonance (EPR, or equivalently, electron spin resonance, ESR) spectroscopy is a highly sensitive and specific technique for investigating melanins [for review, see Sealy et al. (1980)] but has been under-utilized in the study of neuromelanin. Although the latter pigment has been shown by EPR spectroscopy to contain a stable population of organic free radicals which increases upon exposure to visible light (Van Woert et al., 1967; Mikulski, 1970), properties characteristic of more typical melanins (Sarna and Swartz, 1978), these early studies were limited in scope. Since then, little more has been done despite the development of a fairly complete understanding of the paramagnetic nature of melanins in general (Sealy et al., 1980).

Using an established set of EPR criteria for the unambiguous identification and characterization of melanins (Sarna and Swartz, 1978; Enochs et al., 1993a), we have demonstrated recently that human neuromelanin purified from normal substantia nigra is a true melanin (Enochs et al., 1993b). However, compared to purified bovine eye melanin (a typical and fairly well-characterized eumelanin) and various synthetic melanins, it is an atypical melanin that is not well modeled by synthetic dopamine melanin. Some of the unusual features of neuromelanin can be explained by postulating two distinct sources for its free radicals, the dominant one possibly derived from a precursor containing sulfur. This is consistent with recent degradative studies of neuromelanin indicating that it may be a heterogeneous copolymer of dopamine and cysteine or glutathione (Carstam et al., 1991; Zecca et al., 1992).

We also have demonstrated by EPR spectroscopy the presence of significant amounts of paramagnetic metal ions in unprocessed human substantia nigra, part of which at least are bound to neuromelanin in situ (Enochs et al., 1993b). Although other physicochemical studies have shown that this brain region grossly contains large quantities of Fe³⁺, Cu²⁺, and Mn²⁺ (Warren et al., 1960; Cumings, 1968; Earle, 1968; Larsen et al., 1979; Sofic et al., 1988; Dexter et al., 1989, 1991; Riederer et al., 1989; Uitti et al., 1989; Hirsch et al., 1991), histochemical studies have suggested that the metal ions are localized to storage granules within glial cells and are not endogenous to neuromelanin granules in neurons (Barden, 1971; Hill and Switzer, 1984; Connor et al., 1990; Jellinger et al., 1990; Sofic et al., 1991). However, our data have been confirmed recently by other sophisticated physicochemical techniques (Swartz et al., 1992; Perl and Good, 1992; Jellinger et al., 1992), indicating that histochemical staining reactions for metal ions may not be reliable when the ions are bound to melanin.
Interaction of typical melanins with O$_2$ under physiological conditions and their potential toxicity in vivo

Our unambiguous demonstration that neuromelanin is a true (albeit atypical) melanin containing bound redox-active metal ions has significant pathophysiological implications when the reactivity of typical melanins toward molecular oxygen (O$_2$) and its reduced forms is considered [for review, see Sarna and Swartz (1993)]. In the presence of O$_2$, nonilluminated melanin at physiological pH is a low-level source in vitro of both superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) (Felix et al., 1978; Sarna et al., 1980a; Sarna and Sealy, 1984). Superoxide anion is produced from the univalent reduction of O$_2$ by hydroquinonoid and semiquinonoid subunits in melanin (Table 1, Eqs. 1a,b); although most of this O$_2^-$ is reoxidized to O$_2$ by quinonoid subunits (Eq. 1b), part of it spontaneously dismutates (Eq. 2) or is reduced further by melanin to form H$_2$O$_2$ (Eqs. 3a,b) (Korytowski et al., 1985). This process is accelerated greatly by exposure to ultraviolet (UV) or even visible light (Korytowski et al., 1987). Because H$_2$O$_2$ is relatively inert toward pure melanin at physiologic pH, the majority of it appears in the bulk solution (Korytowski and Sarna, 1990).

When containing bound redox-active metal ions or especially in the presence of simple chelate complexes of these ions, nonilluminated melanin also is a source in vitro of the highly reactive hydroxyl radical (OH·), even at physiologic pH. This occurs through a Fenton-type (Halliwell, 1981) decomposition of endogenous or exogenous H$_2$O$_2$ (Eq. 5a) and, like the production of O$_2^-$ and H$_2$O$_2$, is accelerated by exposure to UV or visible light (Korytowski et al., 1987). Simple chelate complexes of redox-active

---

**Table 1. Reactions between melanins, bound redox-active metal ions, and oxygen and its reduced forms**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Mel-Q$^{2-}$ + O$_2$ ⇌ Mel-Q$^-$ + O$_2^-$/</td>
</tr>
<tr>
<td>b</td>
<td>Mel-Q$^-$ + O$_2$ ⇌ Mel-O + O$_2^-$/</td>
</tr>
<tr>
<td>2</td>
<td>O$_2^-$/ + O$_2^-$/ + 2H$^+$ → H$_2$O$_2$ + O$_2$</td>
</tr>
<tr>
<td>3a</td>
<td>Mel-Q$^{2-}$/ + O$_2^-$/ + 2H$^+$ → Mel-Q$^+$ + H$_2$O$_2$</td>
</tr>
<tr>
<td>b</td>
<td>Mel-Q$^-$ + O$_2^-$/ + 2H$^+$ → Mel-O + H$_2$O$_2$</td>
</tr>
<tr>
<td>4</td>
<td>Chel-M$^m$/ + H$_2$O$_2$ → Chel-M$^{m+}$ + OH$^-$ + OH$^-$</td>
</tr>
<tr>
<td>5a</td>
<td>Mel-M$^m$/ + H$_2$O$_2$ → Mel-M$^{m+1}$/ + OH$^-$ + OH$^-$</td>
</tr>
<tr>
<td>b</td>
<td>Mel$<em>{red}$-M$^{m+1}$/ → Mel$</em>{ox}$-M$^m$/</td>
</tr>
<tr>
<td>6</td>
<td>Mel + OH$^-$ → Mel$_{ox}$</td>
</tr>
</tbody>
</table>

*Mel-Q$^{2-}$/ Hydroquinonoid melanin subunits, Mel-Q Quinonoid melanin subunits, Mel- Q$^-$ Semiquinonoid melanin subunits, O$_2$ Molecular oxygen, O$_2^-$/ Superoxide anion, H$_2$O$_2$ Hydrogen peroxide, OH$^-$ Hydroxyl radical, OH$^+$ Hydroxide anion, H$^+$ Proton, Chel-M$^m$/ Chelate-metal ion complex, Mel-M$^m$/ Melanin-metal ion complex, Mel$_{red}$/ Reduced melanin, Mel$_{ox}$/ Oxidized melanin
metal ions are known to cause an analogous breakdown of \( \text{H}_2\text{O}_2 \) (Eq. 4) (Halliwell, 1981), but this reaction is expected quickly to cease as the ions are oxidized to their higher valencies. By contrast, melanin is a redox polymer which can reduce the oxidized metal ions, leading to cycling of their valencies such that a steady-state flux of OH\(^-\) is possible under conditions of constant \( \text{H}_2\text{O}_2 \) concentration or \( \text{O}_2 \) tension (Eqs. 5a,b) (Korytowski and Sarna, 1990). The vast majority of OH\(^-\) is scavenged immediately at the site of its formation, thus causing peroxidative degradation of the melanin (Eq. 6) (Sarna et al., 1986; Korytowski and Sarna, 1990) while simultaneously limiting damage to other molecules that may be present. Specifically, melanin has been shown to accelerate the rate of production of OH\(^-\) in the presence of \( \text{H}_2\text{O}_2 \) and \( \text{Fe}^{3+} \) or \( \text{Cu}^{2+} \), which occurs because of reduction of \( \text{Fe}^{3+} \) to \( \text{Fe}^{2+} \) or \( \text{Cu}^{2+} \) to \( \text{Cu}^{+} \) (Hintz and Kalyanaram, 1986; Korytowski et al., 1987; Pilas et al., 1988; Korytowski and Sarna, 1990).

Since melanin has a high affinity for metal ions (White, 1958; Bruenger et al., 1967; Potts and Au, 1976; Sarna et al., 1976, 1980b, 1981; Larsson and Tjalve, 1978; Francisz et al., 1980; Lyden et al., 1984; Ben-Shachar et al., 1991) and pigmented tissues have been shown to contain impressive amounts of metal ions bound to it in situ, including a variety of redox-active species (Bowness et al., 1952; Stein, 1955; Horcicko et al., 1973; Szerkeres, 1975; Parkinson et al., 1979; Sarna et al., 1980; Okazaki et al., 1985; Enochs et al., 1993b), the observations above suggest that natural melanin may be a source in vivo of \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), and \( \text{OH}^- \), especially on exposure to UV light. Since these species potentially are highly cytotoxic [for reviews, see Halliwell and Gutteridge (1984)], this appears to contradict the generally accepted role of melanin in photoprotection (Pathak et al., 1976; Kollias et al., 1991). However, it is interesting that in skin and hair, which are most exposed to such illumination, the cells specialized for the synthesis of melanin (melanocytes) transfer their melanin to a cell line destined for terminal inactivation and sloughing (keratinocytes) (Jimbow et al., 1976). Furthermore, melanocytes in both the skin and retina are screened at least partly from UV light by the overlying stratum corneum and epidermis in the first instance and by the cornea, iris, and lens in the second (Boettner and Wolter, 1962; Pathak et al., 1976; Kollias et al., 1991).

Under normal conditions, the potential toxicity of melanin in vivo also may be limited by its occurrence within membrane-limited organelles (melanosomes) (Jimbow et al., 1976). Due to its low solubility in lipids (Rumyantseva et al., 1979) and presumably slow diffusion out of the melanosome, any intramelanosomal \( \text{O}_2^- \) would tend to be reoxidized by melanin or reduced further to \( \text{H}_2\text{O}_2 \), while any \( \text{OH}^- \) generated from the decomposition of intramelanosomal or extramelanosomal \( \text{H}_2\text{O}_2 \) would react immediately with melanin or other contents of the organelle (including its membrane). Thus, the melanosome normally would act as a low-level source mainly of \( \text{H}_2\text{O}_2 \), which then could be scavenged readily by intracellular peroxidases and catalase [for review, see Halliwell (1981)].
The role of neuromelanin in Parkinson’s disease

Based on the paramagnetic characteristics of melanin and its high affinity for metal ions, at least two cytoprotective functions other than photoabsorption have been proposed for natural melanin. One is the intracellular scavenging of extraneous or light-induced radicals (Commoner et al., 1954; Mason et al., 1960; Cotzias et al., 1964; Lukiewicz, 1972; Barbeau, 1984a; Sarna et al., 1986), but this is difficult to reconcile with the presence of a relatively impermeable lipid membrane around the melanosome. Another is the sequestering of redox-active metal ions from the cytoplasm under normal conditions, as recently suggested for melanin in the retinal pigment epithelium (RPE) (Sarna, 1992). By the mechanisms above, however, intense or prolonged illumination (especially at short wavelengths) may cause sufficient structural deterioration of melanin that its capacity for binding metal ions is lost and sufficient secondary peroxidation of the melanosomal membrane that its integrity is disrupted. This could result in leakage of the melanosomal contents, including free metal ions, into the cytoplasm with grave consequences for the cell. A similar but more gradual process also could occur during normal aging of melanosomes and would be especially problematic for postmitotic cells, such as those in the RPE, for which no renewal of melanin occurs (Sarna, 1992).

A model for the accumulation of neuromelanin

Most existing data strongly support an autooxidative mechanism for the accumulation of neuromelanin in catecholaminergic neurons (Van Woert et al., 1967; Maeda and Wegmann, 1969; Nordgren et al., 1971; Das et al., 1978; Graham, 1979; Carstam et al., 1991; Zecca et al., 1992). While our recent results have confirmed that neuromelanin is a true melanin (Enochs et al., 1993b), previous histochemical analyses indicate that it may occur in association with lipofuscin (Van Woert and Ambani, 1974; Barden, 1975, 1981; Barden and Brizée, 1987), and a recent study has shown that it has an intrinsic component of fatty acids (Zecca et al., 1992). As the classic “age pigment,” lipofuscin generally is accepted as a nonbiodegradable product of lipid peroxidation that collects in the lysosomes of postmitotic cells during normal aging [for reviews, see Dolman and MacLeod (1981), Barden and Brizée (1987)]. Therefore, it is reasonable to assume that the accumulation of neuromelanin in catecholaminergic brain nuclei occurs by analogy to lipofuscin elsewhere in the central nervous system.

Because no enzymatic mechanism for its formation has been found, neuromelanin most likely is a consequence of the occasional oxidation of catecholamines (e.g. dopamine in the substantia nigra and norepinephrine in the locus coeruleus). Catecholamines in vitro undergo autooxidation in the presence of \( O_2 \), though inefficiently at physiologic pH (Graham et al., 1978a,b; Sealy et al., 1984), and also enzymatic oxidation in the presence of \( H_2O_2 \) and peroxidase (Okun et al., 1971; Kalyanaraman et al., 1984). Since the normal catabolism of catecholamines by monoamine oxidase and their autooxidation by \( O_2 \) are known sources of \( H_2O_2 \) (Cohen, 1983, 1986; Graham, 1984), the intracellular milieu of catecholaminergic neurons would
predispose toward a nonphysiological oxidation of catecholamines in vivo to their corresponding semiquinones and quinones. However, while these species are relatively reactive electrophiles and thus potentially cytotoxic themselves (Graham et al., 1978b; Kalyanaraman et al., 1987), they normally would undergo prompt reduction to the original catecholamine by antioxidants such as glutathione and ascorbate (Heacock and Laidlaw, 1958; Heacock and Scott, 1959; Tse et al., 1976).

Despite the presence of antioxidative mechanisms in catecholaminergic neurons for maintaining their neurotransmitters in a reduced state, it is expected that this system is not perfect. Some of the semiquinones and quinones formed by the oxidation of catecholamines may escape reduction on occasion and randomly form dimers and oligomers with the original catecholamine (Graham et al., 1978b; Sealy et al., 1984) or become reductively coupled to the cysteiny1 groups of GSH or proteins (Graham, 1978b; Rosengren et al., 1985; Fornstedt et al., 1986, 1989; Kalyanaraman et al., 1987; d'Ischia et al., 1987a,b; Ito et al., 1988) or possibly to other cellular constituents. These products probably could not be catabolized in general and would gradually collect in lysosomes, perhaps copolymerizing with the products of lipid peroxidation to form a type of melanized lipofuscin unique to catecholaminergic brain nuclei. Since neurons are postmitotic cells, the accumulation of neuromelanin, like lipofuscin elsewhere, thus would be a measure of the cumulative exposure (in both duration and degree) of individual neurons to oxidative stress.

This model for the accumulation of neuromelanin and our observation of paramagnetic metal ions bound to it in situ (Enochs et al., 1993b) are consistent with previous suggestions that it is a waste product of dopamine metabolism (Graham, 1978a). Nevertheless, this origin still is compatible with a cytoprotective function for neuromelanin under normal conditions. Catecholaminergic neurons are exposed to unusually high levels of $\text{H}_2\text{O}_2$ because of the enzymatic catabolism and autooxidation of dopamine. The relative stability of $\text{H}_2\text{O}_2$ and its ability to diffuse long distances in cells have led to the proposal that the cytotoxicity of $\text{H}_2\text{O}_2$ ultimately results from its decomposition to $\text{OH}^-$, catalyzed in a Fenton-type reaction by free or loosely bound redox-active metal ions in the cytoplasm and causing site-specific peroxidation of lipids, proteins, and nucleic acids (Halliwell and Gutteridge, 1984). In analogy to melanosomes, neuromelanin granules may sequester such metal ions normally, thus reducing their cytoplasmic concentration and confining the products of their reaction with $\text{H}_2\text{O}_2$ to the granules. Through this process, neuromelanin granules also could complement established mechanisms for scavenging $\text{H}_2\text{O}_2$, such as glutathione peroxidase and catalase (Halliwell, 1981), by acting as scavengers themselves of any excess $\text{H}_2\text{O}_2$ escaping enzymatic degradation.

**Hypothetical pathogenesis for the loss of pigmented neurons during normal aging and in Parkinson's disease**

Based on the model above for the accumulation of neuromelanin, two possibilities are suggested for the pathogenesis of neuronal loss in the sub-
stantia nigra during normal aging and in PD, which are consistent with observations that the most highly pigmented neurons in this brain region are most susceptible to death (Pakkenberg and Brody, 1965; Graham, 1979; Mann and Yates, 1979, 1983; Hirsch et al., 1988) and evidence that this may be due to oxidative stress (Cohen, 1983, 1986; Graham, 1984; Hornykiewicz and Kish, 1986; Fahn, 1989; Halliwell, 1989; Youdim et al., 1989; Martilla and Rinne, 1989; Olanow, 1990, 1992; Gotz et al., 1990; Ben-Shachar and Youdim, 1990; Adams and Odunze, 1991a,b; Jenner, 1992; Jenner et al., 1992; Hirsch, 1992; Dexter et al., 1992; Ben-Shachar et al., 1992). First, neuromelanin may form as an indicator of oxidative stress, reflecting an independent, yet-unidentified source of the stress which is endogenous or exogenous to catecholaminergic neurons and generates neuromelanin at a proportionate rate. Second, neuromelanin itself may contribute to oxidative stress as a source of H$_2$O$_2$ when it is intact or of redox-active metal ions if it loses its integrity. More likely still is an interplay between these two roles of indicator and contributor of oxidative stress in which neuromelanin could have a role in its own formation.

According to the model, neuromelanin and lipofuscin normally form early in life as the result of independent oxidative stressor(s) and imperfect antioxidative defense mechanisms, thus accumulating gradually with age. Regardless of whether neuromelanin has a cytoprotective function, the formation of both pigments is expected to accelerate in specific neurons experiencing chronic or repeated oxidative stress. Whereas both neuromelanin and lipofuscin granules also contain paramagnetic metal ions bound to the pigments in situ (Vistnes et al., 1983; Enoch et al., 1993b), however, only the former is susceptible to peroxidative degradation and leakage of its potentially toxic contents into the cytoplasm. This is analogous to melanosomes but would occur by exposure to extragranular H$_2$O$_2$ from the metabolism of dopamine rather than to intramelanosomal H$_2$O$_2$ generated by illumination. Since neurons are postmitotic and neuromelanin apparently does not undergo turnover (Lindquist et al., 1987), such degradation could occur gradually with normal aging of the oldest granules or acutely by a sudden oxidative insult. Over time, the contribution of neuromelanin to oxidative stress thus could result in a positive-feedback cycle leading to a condition of chronic oxidative stress with increased degradation of metal-laden old neuromelanin, eventually overwhelming antioxidative defense mechanisms, with an acute crisis ending in neuronal death.

Such a positive-feedback cycle of increasing oxidative stress not only provides a plausible explanation for the loss of pigmented neurons in the substantia nigra at advanced ages and in idiopathic PD but also is compatible with suggestions that the latter is a form of accelerated aging in this brain region (Barbeau, 1984b; Langston, 1989; Wolters and Calne, 1989). During normal aging, neuronal death occurring sporadically with age in relatively few vulnerable neurons, though progressing somewhat faster at advanced ages, would not cause a functional deficiency of dopaminergic neurons during the average human lifespan since symptoms do not appear until more than 80% of the neurons are lost (Bernheimer et al., 1973). However, chronic oxidative stress in the substantia nigra as a whole would
accelerate this process, and a single intense oxidative insult or repeated smaller insults could lead to the simultaneous degradation of many neuromelanin granules with the acute loss of a large number of neurons. Whether parkinsonism subsequently developed during life would depend on: the density of neurons in the substantia nigra at birth; the age of the individual (determining the number of remaining neurons, the average neuronal content of neuromelanin, and the efficacy of antioxidative defense mechanisms (Fahn, 1989) when the stress began or the insult occurred; and the duration and intensity of the stress or insult. Furthermore, an acute oxidative insult occurring remotely and causing a subcritical depletion of neurons (less than 80%) eventually could lead to parkinsonism because of chronic stress in the remaining neurons secondary to compensatory overactivity (Hornykiewicz, 1966; Bernheimer et al., 1973; Fornstedt et al., 1989), with a more rapid decline in their number than previously.

The proximate trigger(s) for the pathogenesis of idiopathic PD according to the hypothesis above is not specified. Certainly any substance or process would be a candidate if it raised the intraneuronal concentration of \( H_2O_2 \) or independently altered the structure or composition of the neuromelanin granule, making it more susceptible to degradation or otherwise decreasing its postulated cytoprotective ability. One strong possibility consistent with the latter is suggested by the increased content of iron observed in the postmortal substantia nigra of patients with PD (Dexter et al., 1992; Riederer et al., 1992; Ben-Shachar et al., 1992; Perl and Good, 1992; Jellinger et al., 1992) and the greater incidence of this disease in industrial areas where exposure to heavy metals is high (Tanner, 1989; Goldsmith et al., 1990). Elevated levels of redoxactive metal ions in the microenvironment of the neuromelanin granule could lead to saturation of neuromelanin with these ions and the sequelae above. Our hypothesis thus provides for a multifactorial etiology for PD but one in which neuromelanin has a central, active role in its pathogenesis.  

**Future directions**

Additional information is required fully to evaluate our model for the accumulation of neuromelanin and our hypotheses that it has a cytoprotective function under normal conditions but a cytotoxic role in the pathogenesis of PD. This includes: a more precise determination of the nature of purified neuromelanin and its physicochemical properties, including any changes associated with aging or PD; the identification and quantitation of the types and amounts of metal ions actually contained within intact neuromelanin granules and any age- or disease-related differences in them; and a determination of the reactivities of genuine neuromelanin towards \( O_2 \) and its reduced forms, both with and without its normal complement of metal ions, and how these reactivities compare with those of more typical melanins. Elucidating the physicochemical structure of neuromelanin also would lead to a more complete understanding of the mechanism of its formation and would facilitate the development of a synthetic model pigment for use in
The role of neuromelanin in Parkinson's disease

extensive investigations of its reactivity in vitro and, using cultured cells, its potential toxicity in vivo.

With such information and a validation of our model and hypotheses, methods for the diagnosis of PD at a subclinical stage might be further advanced, such as using magnetic resonance or PET imaging of the midbrain to detect subtle changes in the anatomical structure, chemical composition, or metabolism of the substantia nigra (Olanow, 1992; Eidelberg, 1992). Furthermore, novel rational approaches to medical therapy, prophylaxis, or prevention at this early stage might be devised to complement the successful uses of L-dopa and the monoamine oxidase B inhibitor deprenyl in symptomatic PD (Hornykiewicz, 1973; Parkinson Study Group, 1993). Although a recent study indicates that administration of alpha tocopherol does not slow the progression of established PD (Parkinson Study Group, 1993), the success of an approach using ascorbate (Fahn, 1989) or other antioxidants (Hall, 1992) or one using metal chelators such as deferoxamine (Shoulson, 1992) would be consistent with our proposals.

Acknowledgements

The authors gratefully acknowledge the support of this research by the Parkinson's Disease Foundation, the United Parkinson's Disease Foundation, the American Parkinson's Disease Foundation, and the Polish National Committee for Scientific Research (grant KBN-2225).

References

Adams JD, Oduzne IN (1991a) Biochemical mechanisms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity: could oxidative stress be involved in the brain? Biochem Pharmacol 41: 1099–1105