the survival curve, rather than a strictly dose-modifying effect of glutathione removal as seen in the present study.

Since endogenous reducing agents (the main one is glutathione) act in competition with electron-affinic radiosensitizers in the cell, a modest depletion of intracellular glutathione might make the hypoxic cells more susceptible to radiosensitization by these electron-affinic agents. We tested this hypothesis by depleting glutathione in hypoxic CHO cells to varying degrees with DEM and irradiating in the presence of various concentrations of misonidazole. In Fig. 2 the open symbols show the ER produced by different concentrations of MIS alone in hypoxic CHO cells. The closed symbols show that the radiosensitization of hypoxic cells by MIS is enhanced by glutathione depletion: less MIS is needed to produce equivalent radiosensitization in glutathione-depleted cells. Even a concentration of DEM ($2.5 \times 10^{-5} M$) which depletes glutathione to 35 percent of control values, and which itself produces no radiosensitization, reduces the MIS concentration necessary to achieve a given ER by a factor of approximately 3. This factor increases to approximately 15 at $5 \times 10^{-5} M$ DEM, which depletes glutathione to 10 to 15 percent of control values.

We carried out similar experiments in vivo with EMT6 tumors in BALB/c mice (*5*). An ER of 1.5 ± 0.2 for hypoxic EMT6 tumor cells in vivo was achieved by glutathione depletion to 20 percent of control values with DEM (720 mg/kg). This is comparable to the in vitro results with CHO cells at the same degree of glutathione depletion. The combination of DEM and MIS (25 mg/kg) in hypoxic EMT6 tumors in vivo resulted in an ER of 2.0 ± 0.2. This dose of MIS alone did not produce any significant radiosensitization (ER = 1.08 ± 0.05). For MIS alone to produce an ER of 2.0, a dose of 250 mg/kg is required—that is, ten times the dose used in the combination.

The clinical effectiveness of MIS has been severely limited by its neurotoxicity (doses are limited to 20 to 30 mg/kg when given with daily fractionated radiotherapy) (*2*). Our results suggest that the effectiveness of MIS in the treatment of cancer by radiation may be improved by combining it with drugs that deplete intracellular glutathione.

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Eumelanins and Pheomelanins: Characterization by Electron Spin Resonance Spectroscopy

Abstract. *Synthetic dopa melanin and cysteinyldopa melanin have different electron spin resonance spectra. Data are reported for mixtures of these melanins and for dopa-cysteinyldopa copolymers, which are spectroscopically similar. A simple parameterization of the spectra allows estimation of the relative amounts of (i) dopa melanin and cysteinyldopa melanin in mixtures and of (ii) dopa and cysteinyldopa incorporated into copolymers. Several natural eumelanins and pheomelanins have been characterized and shown to be copolymers.*

Mammalian melanin pigments can be classified as either eumelanins or pheomelanins. Both classes are amorphous, heterogeneous polymers (*1*). The major units in eumelanins are thought to be derived from tyrosine through dopa (*2*), while those in pheomelanins are apparently derived from the dopa metabolite 5-*S*-cysteinyldopa (*3*). However, since the sulfur content of many natural melanins is intermediate between that of pure dopa and cysteinyldopa melanins (*4*), it has been argued (*5*, *6*) that copolymers of dopa and 5-*S*-cysteinyldopa are widespread. In support of this, cysteinyldopa-derived units have been chemically identified among the degradation products of eumelanins extracted from mammalian eyes (*7*). The chief criterion for distinguishing between pheomelanins and eumelanins is their solubility in alkali: eumelanins are insoluble in dilute alkali, whereas pheomelanins are alkali-soluble. Some other means of characterizing melanins that reflects the copolymeric nature of most of these materials is desirable.

We showed previously (*8*) that pure cysteinyldopa melanin has a characteristic electron spin resonance (ESR) spectrum associated with a novel kind of biological free radical, probably a semiquinonimine. This spectrum, which has three distinct features (associated with interaction with a nitrogen atom), is different from the single-line, featureless

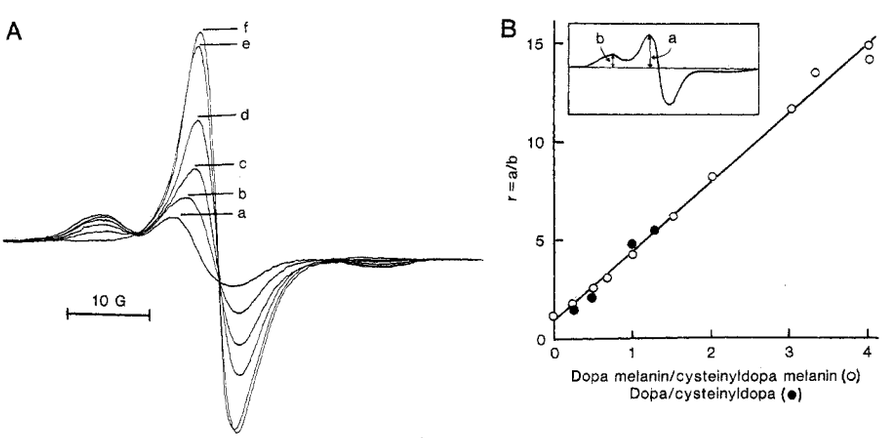

Fig. 1. (A) ESR spectra of mixtures of cysteinyldopa and dopa melanins. Spectra *a* to *f* correspond to mixtures containing 100, 80, 60, 40, 20, and 0 percent (by weight) cysteinyldopa melanin, respectively. Spectra were obtained as previously described (*8*) from suspensions (~ 1 mg/ml) containing 3 m*M* Zn^{2+} (*p*H 4.5) at −196°C. Cysteinyldopa was prepared by the method of Agrup *et al.* (*16*) and melanins by the method of Ito *et al.* (*13*). Melanins were obtained and stored as aqueous suspensions. They were not subjected to drying. (B) Characterization of ESR spectra of mixtures (○) and copolymers (●) in terms of *r* values (see inset).

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spectrum typical of semiquinones in dopa melanin (*9*). Preliminary data suggested that the semiquinonimine species is also present in natural pheomelanins. In one case (pheomelanin from human red hair), a spectral component suggestive of semiquinone radicals was also present, a result which indicated that this material might be a copolymer of cysteinyldopa and dopa. The result also suggested that it might be possible to characterize natural melanins on the basis of their ESR spectra. We now report a simple spectroscopic procedure for characterizing melanins and determining the approximate relative amounts of materials in mixtures and copolymers.

We studied mixtures of dopa melanin and cysteinyldopa melanin and copolymers from the enzymatic oxidation of dopa-cysteinyldopa mixtures. These studies were greatly facilitated by the use of Zn^{2+} ions to enhance radical concentrations. Radicals in melanins form chelate complexes when treated with complexing metal ions at a *p*H of about 5 (*8, 10*), with associated increases in total radical concentration apparently resulting from a shift in a redox equilibrium. Figure 1A shows spectra obtained from pure cysteinyldopa (*11*) and dopa melanins (peaks *a* and *f*, respectively) and from several mixtures of the two materials. The mixtures have features characteristic of the two components, with the cysteinyldopa-melanin feature increasing as the proportion of this material in the mixture is increased. Isoclinic points (three in all) are present as expected for a two-component linear system (*12*).

Figure 1A also indicates that the outermost features of the cysteinyldopa melanin semiquinonimine spectrum are free from the single-line feature of the dopa melanin semiquinone spectrum. Furthermore, in the usual first-derivative display the maximum of the semiquinone signal is located at a crossover in the semiquinonimine spectrum. This implies that for the mixtures the ratio of the two components should, over a range of conditions, be simply related to the ratio of the height of the central feature (*a*) to the height of either the low-field (*b*) or the high-field feature (see inset to Fig. 1B). Because the low-field feature is more intense and can be measured with greater accuracy, it is a more useful parameter than the high-field feature. We used the ratio $r(= a/b)$ to characterize spectra.

In Fig. 1B r is plotted against the ratio (by weight) of dopa melanin to cysteinyldopa melanin in the mixture. A linear relation is obtained over the range of mixtures investigated.

Spectra from the mixtures were compared with those from copolymers of dopa and cysteinyldopa synthesized by enzymatic oxidation of mixtures containing 20, 33, 50, and 56 percent dopa by weight. Sulfur analysis showed that cysteinyldopa and dopa were incorporated into the polymers in the ratios in which they were present in the initial oxidation mixtures (*13*). The ESR spectra of the copolymers closely resemble those of simple mixtures (Fig. 1A), except that the separation of the extrema ($2A_z$) in the spectra of the semiquinonimine is reduced by about 3 percent (to about 32 G compared with the 33 to 33.5 G typically measured for pure cysteinyldopa melanin) (*8, 14*).

For the copolymers, r is linearly related to the ratio of the amounts of dopa and cysteinyldopa in the polymerization mixture. Data points (closed circles in Fig. 1B) fall on the straight line obtained for the mixtures. Therefore, measurement of r can, with the use of Fig. 1B, be used to estimate either the relative amounts of dopa melanin and cysteinyldopa melanin in mixtures of the two, or the relative amounts of dopa and cysteinyldopa in mixtures that were copolymerized. The best fit to all the data in Fig. 1B gives $r = 1.2 + 3.5$ (D)/(CD), where (D)/(CD) is the ratio (by weight) of dopa melanin to cysteinyldopa melanin (for mixtures) or of dopa to cysteinyldopa (for copolymers). The intercept of this plot is the r value (1.2 ± 0.1) for pure cysteinyldopa melanin.

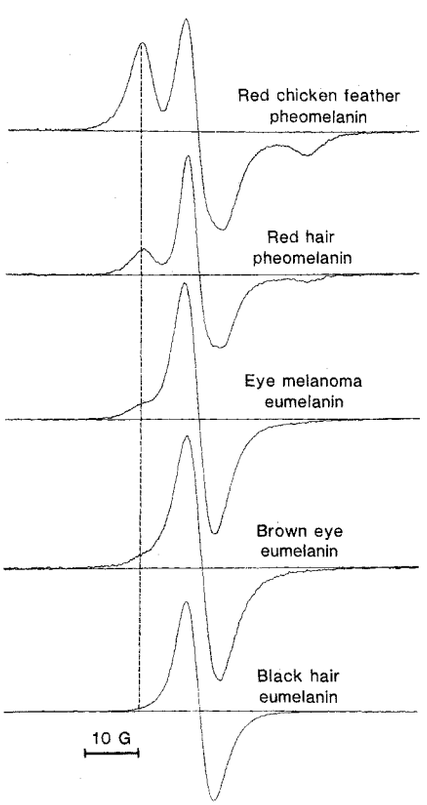

Fig. 2. ESR spectra from suspensions of natural melanins (~ 1 mg/ml) containing 3 m*M* Zn^{2+} (*p*H 4.5) at −196°C. Chicken feather and hair melanins were extracted by the method of Bolt (*18*), except that extraction was carried out with 1*M* NaOH under nitrogen. Eye melanins were extracted as described previously (*19*). As with the synthetic materials, natural melanins were not dried but were obtained and stored as aqueous suspensions.

We also investigated natural melanins from several sources: melanin extracted from red and black human hair, from red and black chicken feathers, from human brown eyes and an eye melanoma, and from B-16 melanoma cells. Of these, the melanins from red hair and chicken feathers are pheomelanins (on the basis of their solubility in dilute alkali) and the others are eumelanins.

Electron spin resonance spectra of several of these materials are shown in Fig. 2. A gradual change in ESR properties occurs over the series from the red chicken feather pheomelanin to the black hair eumelanin. The spectrum of the red chicken feather pigment ($r = 1.3$) is close to that of pure cysteinyldopa melanin ($r \simeq 1.2$). This r value suggests that red chicken feather pheomelanin is a polymer with > 90 percent cysteinyldopa. In contrast, the pheomelanin extracted from red hair has a pronounced central feature and $r = 4.5$. This corresponds to a copolymer with approximately equal amounts of cysteinyldopa and dopa. (The solubility of the materials in alkali rules out the possibility that they are mixtures of dopa and cysteinyldopa melanins.)

The remaining spectra shown are of eumelanins. Two of them—spectra obtained from an eye melanoma and brown eye melanin—again show indications that the materials are copolymers. Thus the shoulder at low field (more prominent in the spectrum of the eye melanoma) appears at the field position expected for a dopa-cysteinyldopa copolymer. Approximate r values for the eye melanoma and brown eye melanin are 8 and 12, respectively, corresponding to copolymers with about 32 and 24 percent cysteinyldopa if the data for the copolymers in Fig. 1B are extrapolated to higher values of r. These percentages may slightly overestimate the amounts of cysteinyldopa in the melanins (overlap of radical spectra in the copolymers becomes more significant for high values of r), but are of the order expected based on the high sulfur content reported for melanin from human brown eyes (*7*). The spectrum of black hair melanin showed little evidence that it is a copolymer, nor did the spectra of melanins from black

chicken feathers and from B-16 melanoma cells, which were similar to the spectrum of black hair melanin.

Our data thus support the suggestion (*5, 6*) that many natural melanins are copolymers of dopa and cysteinyldopa. Such copolymers may conveniently be characterized in terms of an *r* value, which is related to the proportions of dopa and cysteinyldopa incorporated into the polymer. We have shown that pheomelanins from red chicken feathers and red hair are spectroscopically dissimilar and that red hair pheomelanin is a copolymer. The argument that some eumelanins are also copolymers, which was based largely on the high sulfur content of some of these materials (*5, 6*), is supported by our spectroscopic evidence that human brown eye melanin is such a copolymer. It seems that structures characteristic of pheomelanin can be found in tissue other than hair or feathers. It should be possible to use the ESR method to test for the presence of pheomelanin in skin, which is suggested by morphological studies (*2*) but has yet to be chemically verified. This is important in view of a putative link (*15*) between the high incidence of skin cancer in red-haired individuals and the ultraviolet-induced breakdown of pheomelanin presumed to be present in the skin.

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Variation in the Social Grouping Tendency of a Communal Web-Building Spider

Abstract. *The orb web–building spider,* Metepeira spinipes, *from Mexico occurs solitarily and, more frequently, in aggregations of 5 to 150 or more individuals. Although communal, individuals maintain webs and retreats within the colony and capture their own prey. Group size and interindividual distance apparently vary in response to climate and availability of prey.*

Spiders are usually solitary, exhibiting aggressive behavior toward other animals, including conspecifics. Communal and cooperative living patterns have been observed in a few species from several families (*1–3*). Group living patterns in spiders range from simple temporary aggregations to communal web-building, cooperative prey capture, and indiscriminate brood care (*3, 4*).

Metepeira spinipes Pickard-Cambridge, an orb web–building spider, may be solitary, but more frequently lives in permanent aggregations of 5 to 150 or more individuals (*5*). Observations of this species in central Mexico in 1978 and 1979 showed that it exhibits a communal-territorial living arrangement; that is, individuals maintain their own webs and retreats within a colony and capture their own prey (*2, 6*). Aggregations of *M. spinipes* remain together for long periods of time (*7*), and individuals taken from colonies hundreds of miles apart may build webs together (*8*). The social grouping tendency of this species appears to be flexible; group size and interindividual distance may vary as a function of environmental severity and the relative availability of prey. The combination of solitary and communal behavior exhibited by this species suggests that it may represent an intermediate stage in the evolution of social behavior in spiders.

The web of individual *M. spinipes* is characteristic of the genus: a three-dimensional space web and catching spiral composite with a retreat in the space web. Signal threads connect the hub of the spiral and the retreat, where the spider rests (*5*). In the groups observed individuals had their own retreats and spirals, but space webs were joined (*2, 5, 6*). The sticky catching spirals are taken down and renewed daily, but the communal space web, which serves as a framework for the web-building activities of numerous individuals, is left intact. The communal web is a mass of interconnected webbing that is attached to the vegetation, usually *Agave* and *Opuntia*. Although the communal web is built and maintained by the efforts of all colony members, individuals defend their spirals and retreats against intruders and interact while on the space web. Prey captured on spirals are not shared.

Group size in *M. spinipes* varies by habitat (Fig. 1). In those where prey availability is low and environmental conditions are extreme, such as a high-altitude habitat (Parque Sierra Morelos) or a desert grassland (San Miguel de Allende), individuals are predominantly solitary or live in small groups. In intermediate sites such as agricultural areas with seasonal rainfall (Toluca, Tepotzotlán, and Guadalupe Lake) spiders occur more frequently in aggregations. Differences in group size between these sites appear to be attributable to availability of prey. In the moist tropical site, where climate is favorable all year and insect abundance is great, colony size is large (*9*). The distributions of group size in all but one habitat we studied (Parque Sierra Morelos) were significantly different ($P < .05$) from a Poisson distribution truncated at zero, indicating nonrandom clumping at large colony sizes (*2, 6*).

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