becomes available. Iron catalyzes the metabolism of oxygen radicals (O_2^-) to hydroxyl radicals (OH^-) and subsequent lipid peroxidation. These can damage cell and organelle membranes and lead to inflammatory damage of tissues. Vierucci *et al.* (*13*) showed that HBV proteins stimulate the production of oxygen radicals by phagocytic cells without being phagocytized. Also, the presence of the iron can attract inflammatory cells that have receptors for iron-binding proteins. These processes can contribute to the gradual destruction of the cells replicating virus and containing saturated ferritin and may thus increase the selective advantage of the cells that are not replicating HBV or that do not have saturated ferritin.

Persistent HBV infection may result in increased iron stores and be beneficial to individuals with iron-deficient diets. Therefore, early detection or prevention of PHC based on a monitoring of iron balance in carriers might serve to supplement the use of α-fetoprotein and other diagnostic aids.

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The Radicals in Cigarette Tar: Their Nature and Suggested Physiological Implications

Abstract. *The paramagnetism of cigarette tar is found to be associated with at least four different types of species. One of the types is responsible for over 80 percent of the total paramagnetism and has a signal intensity that is independent of temperature from 60 to 250 K. This non–Curie-Weiss temperature dependence indicates that the principal paramagnetic species in tar is not an organic monoradical (doublet) species but instead is a donor-acceptor excimer with a paramagnetic excited state and a diamagnetic ground state. Modeling experiments suggest that the excimer consists of quinone (Q) and hydroquinone (QH_2) molecules held in a tar matrix. Since such Q-QH_2 species are catalysts for the oxidation of hydrocarbons and are very active redox systems, this paramagnetic species may be implicated in the cocarcinogenic properties of tar. Alternatively, since semiquinone radicals are known to bind to DNA, the tar paramagnetic species may be directly involved in the carcinogenic properties of tar.*

Cigarette tar has a long-lived paramagnetism, first detected by electron spin resonance (ESR) techniques by Ingram and his co-workers in 1958 (*1*). These researchers suggested (*1*) that the tar radical is an odd electron delocalized onto a large polynuclear aromatic hydrocarbon (PAH). In the intervening 25 years, virtually no progress has been made toward understanding the structure or possible physiological effects of this tar radical (*2, 3*). We have initiated a systematic study of the nature of the radicals in cigarette smoke and tar, and we present here data that indicate structures and unexpected paramagnetic properties for the tar radical (*4*).

Studies (*5, 6*) of the temperature and saturation behavior of the ESR signal of tar demonstrate that it contains at least

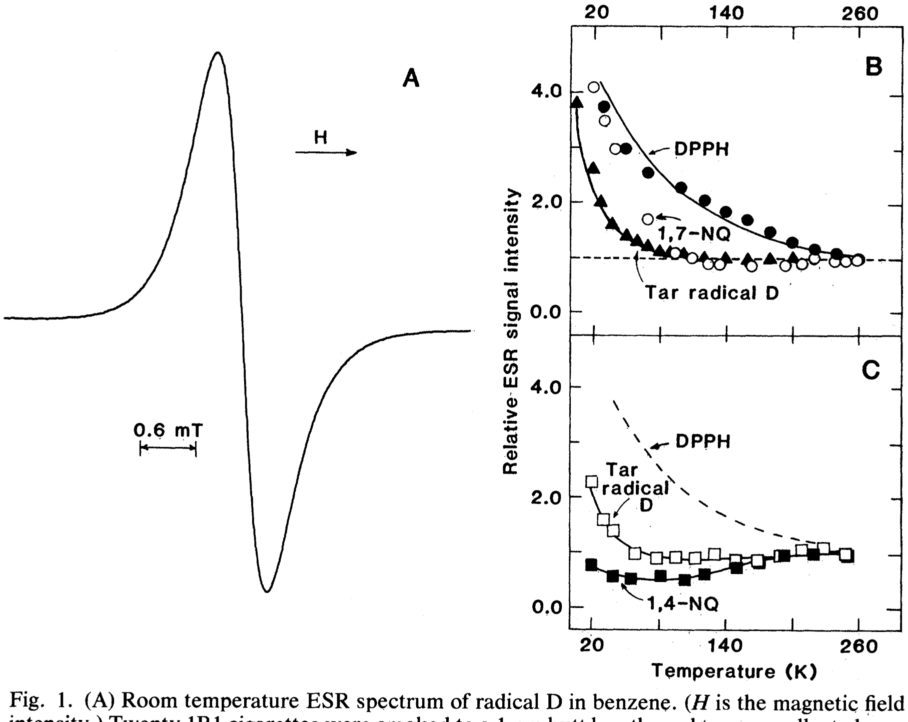

Fig. 1. (A) Room temperature ESR spectrum of radical D in benzene. (*H* is the magnetic field intensity.) Twenty 1R1 cigarettes were smoked to a 1-cm butt length, and tar was collected on a Cambridge filter positioned 5 cm from the cigarette. The radical was then extracted from the tar with 20 ml of benzene. The spectrum was recorded on a Bruker ESR 100 D spectrum interfaced to an ASPECT 2000 computer. The spectral parameters were as follows: microwave frequency, 9.4216 GHz; power, 2 mW; modulated amplitude, 0.2 mT; modulated frequency, 100 kHz; time constant, 1 second; scan rate, 0.01 mT/sec. Identical spectra were observed for radical D in benzene and for the solid residue after the benzene had been evaporated. (B) Relative ESR signal intensity (normalized to the intensity at 250 K) in the temperature range 20 to 250 K of solid tar radical D after the evaporation of the benzene solvent (▲), solid DPPH (●), and solid 1,7-NQ (○). The temperature was regulated with a helium transfer system (Air Products, model LTD-3-110), and signal intensities were calculated by double integration of the first-derivative spectrum with the ASPECT 2000 computer. (C) Same as (B) except that tar radical D and 1,4-NQ are in benzene.

four different types of paramagnetic species with a total paramagnetism of ~ 6 × 10^{14} spins per cigarette or 3 × 10^{16} spins per gram of tar. The four paramagnetic species are as follows: radical A, an inorganic phosphorus radical (7); radical B, graphitic carbon with a *g*-factor = 2.0028 and line width $\Delta H_{\text{p-p}}$ = 0.10 mT (8); radical C, a PAH radical with *g*-factor = 2.0026 and $\Delta H_{\text{p-p}}$ = 0.2 mT (9); and radical D, quinone-hydroquinone systems with *g*-factor = 2.0035 and $\Delta H_{\text{p-p}}$ = 0.66 mT. We report here the nature of the principal radical in tar, radical D, which represents more than 80 percent of the total tar paramagnetism, and suggest possible roles for this radical in the carcinogenic and cocarcinogenic properties of tar.

We have found, contrary to the result reported by Rowlands *et al.* (3), that radical D can be extracted into organic solvents. Tar was immediately extracted with benzene and filtered twice to produce a clear reddish-brown solution. Vacuum-evaporation of the benzene from this solution gives radical D as a solid phase. (All work was performed at room temperature under dim illumination, and ESR spectral data were recorded directly after sample preparation to avoid possible effects of aging.) The ESR spectrum of radical D in benzene or as a solid at room temperature shows the presence of a single, symmetrical Lorentzian line (Fig. 1A). In the dark and in the presence of air, radical D in benzene has a half-life of ~ 12 days; however, it has a much shorter half-life in the presence of light. Obviously, the species giving rise to this signal is not a PAH radical, which would be very short-lived and would have a *g*-factor (*10*) in the range 2.0025 to 2.0029. The lifetime and *g*-factor are consistent with a radical in which the unpaired electron is delocalized over a large structure containing heteroatoms (*11*), for example, as in semiquinone radicals.

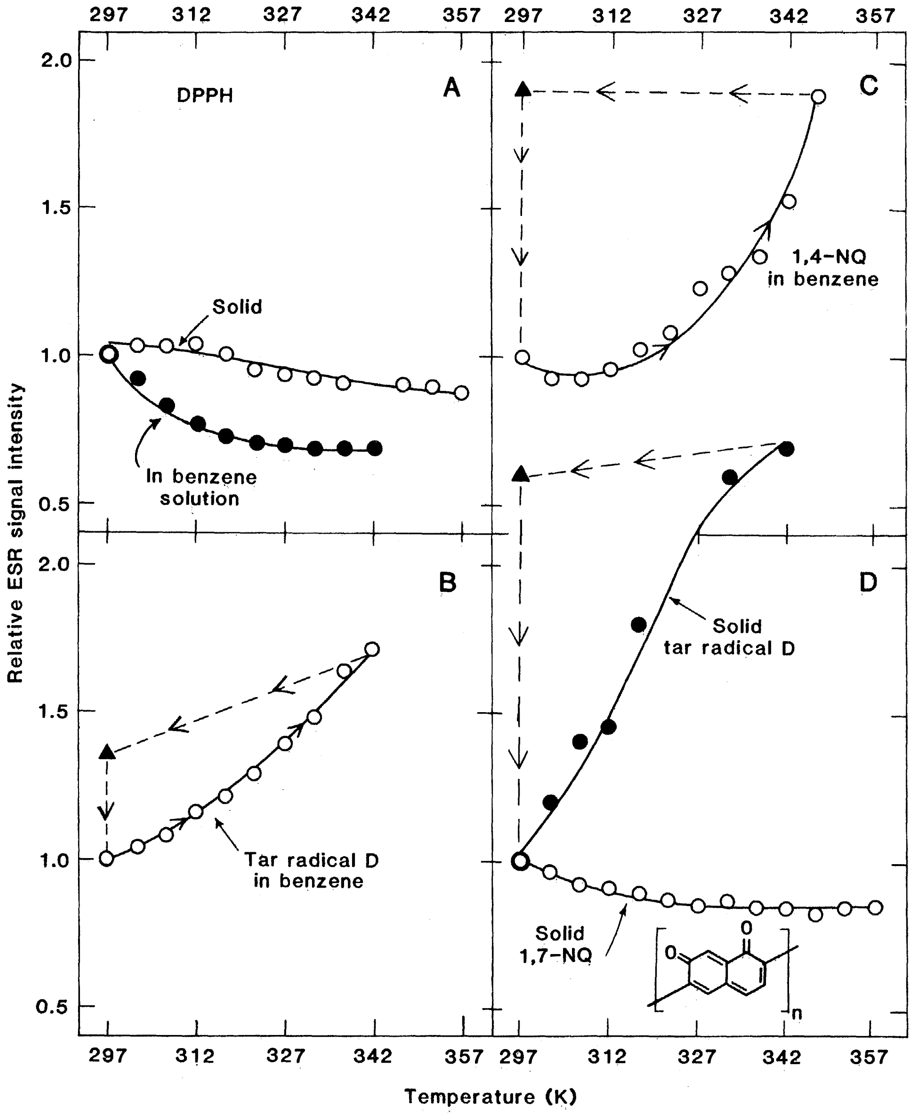

Fig. 2. Relative ESR signal intensity (normalized to the intensity at 297 K) over the temperature range 297 to 357 K of (A) solid DPPH (○) and benzene-solubilized DPPH (●); (B) tar radical D in benzene extract; (C) 1,4-NQ in benzene (○); and (D) solid 1,7-NQ (○) and solid tar radical D (●). Intensities were determined by double integration as in Fig. 1. Arrows along the dashed lines in (B, C, and D) show the direction of temperature change used to observe the hysteresis of the signal intensity; triangles indicate the signal intensities at 297 K after heating first to 342 K.

To further probe the nature of tar radical D, we have recorded its spectrum from 20 to 342 K, determining line shape, line width, *g*-factor, and signal intensity at each temperature setting. In order to measure any change in each parameter, the spectrum of solid α,α-diphenyl-β-picrylhydrazyl (DPPH) was recorded over the same temperature range.

Figure 1, B and C, shows the variations in signal intensity in the low-temperature region (20 to 250 K) for radical D in benzene and as a solid and for solid DPPH. Over this temperature range, there is no *g*-factor or line-width variation in the spectrum of radical D. The line width and the *g*-factor of radical D in benzene also are unaffected when the solution is saturated with O_2 or is degassed.

For temperatures above 20 K, the signal intensity of DPPH decreases with increasing temperature in a behavior that is predicted by the Curie-Weiss law for a system consisting of a single paramagnetic state (*12*) with no low-lying excited states (either diamagnetic or paramagnetic) that can be thermally populated easily. This behavior is in direct contrast to that observed for radical D.

The lack of temperature dependence of the signal intensity of radical D in the region from 60 to 250 K implies that its paramagnetism is associated with an excited diradical or triplet state rather than the ground state. This explanation is similar to that suggested to explain the paramagnetism observed in coal and petroleum asphaltenes (*13*). The single structureless line at high temperatures is similar to the spectra observed for some charge-transfer complexes (*14*) and implies that any spin-spin interactions in the paramagnetic excited state are small.

Charge-transfer complexes also have been proposed to explain the paramagnetism in several biological quinone-hydroquinone (Q-QH_2) materials such as humic acids (*15*) and melanins (*16*). Since the reported paramagnetism of these systems is very similar to that of radical D, radical D may also be a Q-QH_2 system. The amount of radical D in tar is increased if the cigarettes are enriched (5) with quinones (like naphthoquinone) but is unaffected by enrichment with PAH (such as pyrene and anthracene); these results imply that it is the Q-QH_2 properties of tar that give radical D its characteristic paramagnetism.

In an effort to model the ESR behavior of tar with synthetic Q-QH_2 systems, we have polymerized quinones (*5*), using two different techniques. In the first, 1,4-naphthoquinone was dissolved in 20 ml of benzene and the solution was allowed to stand at 95°C in the presence of air for 2 hours. During this period the quinone undergoes a random polymerization, and the color of the solution turns dark reddish-brown, much like that of the tar extract in benzene. This polymer (1,4-NQ) is benzene-soluble and exhibits a paramagnetism possessing a single-lined, Lorentzian ESR spectrum with g-factor = 2.0035 and line width $\Delta H_{\text{p-p}}$ = 0.36 mT. In a second technique, 1,7-dihydroxynaphthalene was polymerized by the technique of Iwasawa *et al.* (*17*) to give a polymer identified by them as poly-1,7-naphthoquinone (1,7-NQ). This polymer (structure shown in Fig. 2D) is insoluble in benzene and also exhibits paramagnetism (7×10^{18} spins per gram) consisting of a single Lorentzian line with a g-factor = 2.0035 and $\Delta H_{\text{p-p}}$ = 0.57 mT. Figure 1, B and C, shows the temperature dependence of signal intensity of these polymer radicals; like radical D, the ESR signal intensity in these polymers is virtually constant over a large temperature range.

The temperature dependence of the ESR intensity of both radical D and the synthetic Q-QH_2 polymers depends on two factors. The first is the thermal population of the paramagnetic excited state; since the Boltzmann population of this state decreases exponentially with decreasing temperature, the overall magnetization also decreases with temperature. In contrast, the difference in the relative populations of the individual Zeeman levels of the paramagnetic excited state increases with decreasing temperature, producing an increased ESR absorption from the decreasing fraction of paramagnetic molecules as the temperature decreases. Over the temperature range studied, these two effects appear to counterbalance one another, yielding a line whose intensity is temperature-insensitive. In contrast, DPPH shows an increase in magnetization with decreasing temperature, as expected from the Curie-Weiss law for a system with a doublet ground state (that is a free radical).

We also studied the ESR intensity over a higher temperature range where the benzene solvent remains fluid. Figure 2 compares the behavior of radical D, DPPH, and the two model systems over the range 297 to 357 K. Here again, DPPH continues to show a $1/T$ signal intensity (where T is temperature) characteristic of a radical obeying the Curie-Weiss law. However, the signal intensities of both radical D and 1,4-NQ increase with increasing temperature (Fig. 2, B and C). Similar increases in signal intensity with increasing temperature have been observed in melanins (*16*) and in several polymers containing conjugated systems such as polyphenylacetylene and polyphenylene (*18*). In these latter systems (*18*) a hysteresis has been observed in the signal intensity when the temperature is raised and lowered. Similar effects are observable in the spectra of both radical D and 1,4-NQ. Figure 2, B and D, shows that the signal intensity of radical D increases after heating from 297 to 342 K and then cooling back to 297 K. The new intensity decays to the old intensity in a matter of several hours (see arrows along the dashed lines in Fig. 2, B and D). Although it has increased in intensity, the new signal at 297 K retains all the spectral and saturation parameters of the old signal and therefore probably is the original paramagnetic center in increased concentration. The 1,4-NQ radical (Fig. 2C) shows the same hysteresis as observed for radical D. This hysteresis is unaffected by the presence of O_2.

In conclusion, we suggest that the principal tar radical consists of a Q-QH_2 system undergoing charge-transfer interactions; these interactions are modeled very closely by the Q-QH_2 system present in polynaphthoquinones. The data suggest that radical D is a stack or chain of alternating donor and acceptor systems, as in a Wannier triplet exciton (*19*). The donor is very likely a hydroquinone; the acceptor clearly is a quinone.

Cigarette tar is a complete carcinogenic system, containing both procarcinogens (such as benzo[*a*]pyrene) and potent cocarcinogens, the most important of which is catechol [present at up to 50 mg per 100 cigarettes (*20*)]. The redox activity (*21, 22*) of the Q-QH_2 materials in tar and their tendency to form charge-transfer complexes suggests a possible explanation for the cocarcinogenic activity of these materials. The PAH molecules in tar are relatively oxidation-resistant, whereas polyquinones are known (*17*) to be active catalysts for the oxidation of hydrocarbons at relatively low temperatures. If the Q-QH_2 and PAH molecules in tar exist in a charge-transfer complex, the paramagnetic Q-QH_2 system could to act as a cocarcinogen (*23*) by catalyzing the oxidation of the PAH and effecting its conversion to a carcinogenic compound. Alternatively, Nagata *et al.* have shown that semiquinone radicals bind to DNA (*24*). If the Q-QH_2 tar system possesses a similar reactivity, then it itself could contribute to the carcinogenicity of tar.

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