murine bone marrow cultures at 4- to 6-week intervals. Growth in subculture remains vigorous for 1 to 3 months. The line currently being carried has been serially passaged through five marrow cultures in 7 months. Both BALB/cCr and W/W^V marrow has been used successfully to prepare subcultures.

Cultivation of pathogenic trypanosomes with primary cultures of normal host cells offers a means to explore a variety of physiological interactions between host tissues and these extracellular parasites. My results suggest that bone marrow adipocyte-epitheloid complexes that potentiate hematopoiesis may also potentiate trypanosome growth. Three-week-old bone marrow cultures are known to support active hematopoiesis for several months when recharged with fresh marrow as a source of stem cells (*8, 9*). Thus this culture system may also prove useful in elucidating postulated mitogenic or toxic influences of trypanosomes on blood cell precursors (*15*). In addition, it should permit the simultaneous screening of the trypanocidal and marrow-suppressive effects of drugs or other agents in vitro. In this regard, it is significant that human bone marrow can be grown by methods similar to those described here (*16*).

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Serum Ferritin as a Predictor of Host Response to Hepatitis B Virus Infection

Abstract. *With hemodialysis patients, a high serum ferritin before there was serological evidence of hepatitis B virus infection increased the likelihood that the infection would be persistent. This finding suggested that hepatitis B virus is likely to infect and actively replicate in liver cells with the propensity for increased ferritin synthesis. The virus itself could stimulate the synthesis of ferritin in a cyclic positive feedback mechanism that increases intracellular ferritin concentration and, eventually, intracellular iron. Transformed liver cells have low iron content, do not replicate hepatitis B virus, and require iron for growth. Infected, nonmalignant liver cells could supply iron to the transformed cells and nourish their expansion.*

Variation in the response of humans to infection by hepatitis B virus (HBV) is one of the most perplexing aspects of the biology of this virus. Females are more likely than males to make antibody to the viral surface antigen (HBsAg), whereas males are more likely to become carriers and to have HBsAg persisting in their blood. The prevalence of antibody to HBsAg increases with age, whereas surface antigen frequency increases initially and then declines after the teenage years in most populations.

To identify factors that might contribute to the carrier state, we examined males with Down's syndrome because they are highly susceptible to HBV infection (*1*). We measured serum iron, in addition to other variables, because iron is often increased in patients with hepatitis. We found that serum iron and transferrin saturation values were higher in the patients who were carriers than in those who were not carriers. These findings were confirmed in a study of male and female patients undergoing hemodialysis treatment (*2*).

Those studies established that the association of increased serum iron with the HBV carrier state was independent of the increased serum iron resulting from liver cell breakdown as measured by alanylaminotransferase (ALT; formerly referred to as SGPT). However, it was not possible to ascertain whether the increased serum iron was a consequence of HBV infection or, alternatively, whether the carrier state occurred more frequently in individuals with increased serum iron.

In a retrospective cohort study of patients treated with hemodialysis, changes in iron level were measured during a period in which HBV infection was known to have occurred (*3*). Three conditions had to be met for a patient to be included in the study: (i) the patient had to have at least one serum sample that was positive for HBsAg; (ii) the serum sample obtained 2 months before HBsAg was first detected had to be negative for both HBsAg and its antibody; and (iii) a serum sample had to be obtained 12 months after HBsAg was first detected (*4*). Fifty-four patients who met these criteria during the period 1974 to 1981 were included in the analysis. The key finding was that the amount of iron in the serum before the initial finding of HBsAg was no greater in individuals destined to be carriers than in those who proved to be transiently infected.

In blood, iron is transported by transferrin and is stored primarily in the liver and spleen in the form of ferritin. Although most of the ferritin in the body is intracellular, it is found in low concentrations in the serum. In normal individ-

uals, serum ferritin is highly correlated with total body iron stores (*5*). For this reason, the analysis of the hemodialysis patients was expanded to include transferrin and serum ferritin levels.

Data collected before HBsAg was first detected in the serum were used to predict the outcome 12 months after this finding. Two outcomes were predicted: persistent infection or transient infection. Five variables were considered: sex, serum iron, serum ferritin, transferrin, and ALT (*6*).

Table 1 shows a significant difference for mean log serum ferritin values of transiently and persistently infected females ($P < .001$); the corresponding values for males gave $P = .11$. Thus serum ferritin appeared to be the best candidate for improving the simple prediction that males will be persistently infected and females will be transiently infected.

A stepwise linear discriminant analysis was undertaken to search for the best combination of variables that predict outcome. All of the data were combined and, as expected, sex was the most significant variable. In addition, log serum ferritin was also significant ($P < .05$). The best model was $y = 0.412 - 1.931\ S + 1.257\ F$, where $S = 1$ for males, $S = 2$ for females, and F is the log (base 10) of the serum ferritin concentration. Values of $y > 0$ predict that the subject has a persistent infection and $y < 0$ that the subject has a transient infection.

If the mean values of S and F from the last two rows of Table 1 are used in the model, y is 0.68 for carriers and -0.79 for those with transient infections. This suggests a criterion for dividing the population into four groups: (i) those with y values less than -0.79, (ii) those with y values between -0.79 and 0.00, (iii) those with y values between 0.00 and 0.68, and (iv) those with y values greater than 0.68.

The model makes no formal prediction about the presence or absence of antibody to HBsAg. Nevertheless, the patients known to have transient infections can be further partitioned according to their antibody status 1 year after the initial finding of HBsAg (Table 2). Those patients who still had no antibody to HBsAg had y values that were relatively uniformly distributed across the four categories of Table 2, and only five of the ten patients had been correctly predicted to have a transient infection. However, 13 of 15 individuals who had antibody to HBsAg were correctly classified as transiently infected, with the majority having y values less than -0.79. Little is known about the dynamics of the metalloproteins in healthy individuals. Therefore, the observation that an increase in serum ferritin before HBV infection can be used to predict the response to the infection provides a new direction for research.

Birgegard (*7*) proposed that increased serum ferritin during unspecified acute viral infections was the result of augmented synthesis rather than release from damaged cells. The model that we derived suggests that the propensity for augmented synthesis, as indicated by initial serum ferritin levels, can increase cellular susceptibility to persistent HBV infection. Taken together, these two proposals indicate a positive feedback mechanism that results in an increased intracellular ferritin concentration. The augmented synthesis need not be abnormal. Even though the use of normal values in hemodialysis patients is questionable, the mean serum ferritin levels we analyzed were within the normal range.

The attributable risk of persistent infection with HBV to primary hepatocellular carcinoma (PHC) is nearly 100 percent (*8*). We had speculated earlier that transformed liver cells depend on surrounding cells to provide iron for their growth (*3*). This hypothesis was supported by four lines of evidence: (i) cancer cells have much less iron storage than nonmalignant cells, as judged by staining (*9*); (ii) HBV is actively replicated in the normal liver cells of patients with PHC, but not in the transformed cells (*10*); (iii) removal of iron decreases the growth of cells in culture (*11*); and (iv) carriers of HBV have increased serum iron (*1–3*).

Primary hepatocellular carcinoma occurs in areas of the world, like West Africa, where diets are deficient in iron. Sufficient iron to support the rapid growth of tumor cells, when there is no obvious interruption of normal cell function, could be accounted for by the latent period of 20 years or more between HBV infection and PHC. With persistent HBV infection in cells with increased ferritin concentrations, a cellular source for extra iron storage is possible. Carriers would have a heterogeneous distribution of intracellular iron in normal cells, with the largest concentrations in cells that are actively replicating HBV.

Blake *et al.* (*12*) suggested a mechanism by which iron can damage a cell. When moderate amounts of iron are bound to apoferritin, little free iron is available within the cell and its immediate surroundings. When excess iron results in saturation, the ferritin-free iron

Table 1. Data collected 2 months before HBsAg was found in the serum of patients undergoing hemodialysis treatment. F, log serum ferritin (serum ferritin was measured in nanograms per milliliter); ALT, alanylaminotransferase, measured in Karmen units (*14*); S, arbitrary value of 1 for males and 2 for females. Results are given as means ± standard deviations. Only the difference in F values for females was significant at the .05 level. The classification of the infection as persistent or transient was made 1 year after HBsAg was first detected.

Classification	N	Serum iron (μg/dl)	Transferrin (mg/dl)	F	ALT	S
			Males			
Persistent	23	85.0 ± 32.7	309 ± 86.9	1.98 ± 0.62	8.83 ± 3.97	1
Transient	8	87.3 ± 31.4	313 ± 110	1.60 ± 0.37	10.9 ± 6.45	1
			Females			
Persistent	6	132 ± 63.6	310 ± 95.6	2.42 ± 0.57	10.7 ± 10.3	2
Transient	17	98.6 ± 46.4	313 ± 98.1	1.64 ± 0.51	7.24 ± 6.48	2
			All patients			
Persistent	29	94.7 ± 44.0	303 ± 87.0	2.07 ± 0.63	9.21 ± 5.66	1.21
Transient	25	95.0 ± 41.8	313 ± 99.7	1.62 ± 0.46	8.40 ± 6.54	1.68

Table 2. Patients grouped by values of y from the prediction equation $y = 0.412 - 1.931\ S + 1.257\ F$. The classification of the infection as persistent or transient was made 1 year after HBsAg was first detected. The presence of antibody was determined in the 25 patients who proved to have transient infections.

Item	Number of patients in each category			
	$y < -0.79$	$-0.79 \le y < 0.00$	$0.00 \le y \le 0.68$	$y > 0.68$
Classification				
Persistent	2	3	10	14
Transient	14	4	5	2
Antibody				
Absent	3	2	4	1
Present	11	2	1	1

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

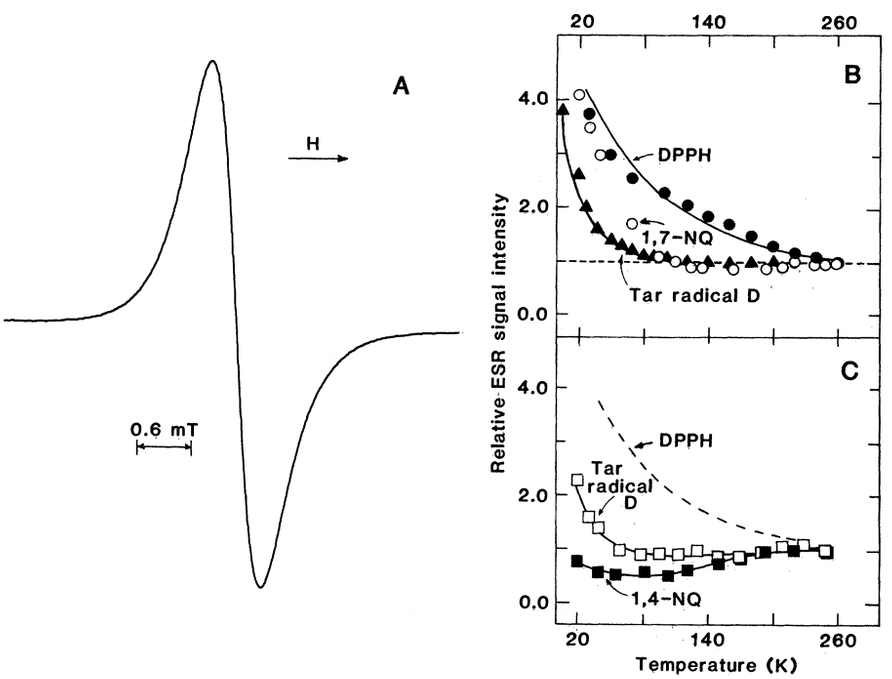

Fig. 1. (A) Room temperature ESR spectrum of radical D in benzene. (*H* is the magnetic field intensity.) Twenty 1R1 cigarettes were smoked to a 1-cm butt length, and tar was collected on a Cambridge filter positioned 5 cm from the cigarette. The radical was then extracted from the tar with 20 ml of 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.