Ferromagnetism and EMFs

SIR — The question of whether weak, extremely low-frequency electromagnetic fields (EMFs) can cause cancer always generates heated debate (see, for example, refs 1–3). In addition to epidemiological studies, a substantial body of literature exists on EMF stimulation of cells grown *in vitro* (for example, refs 4, 5). Although numerous effects have been reported, many have been difficult to replicate (see refs 6, 7), and no clear biophysical mechanism has emerged. Many of the proposed mechanisms, like ion cyclotron resonance[8], have drawn criticism for being physically unrealistic (see ref. 9).

From developments in a totally unrelated field, there may be a much simpler, as yet overlooked, mechanism for explaining many of these *in vitro* EMF cellular effects. For the past two decades, the study of the biologically precipitated ferrimagnetic mineral magnetite (Fe_3O_4) has relied heavily on the use of ultrasensitive superconducting quantum interference device (SQUID) magnetometers to quantify trace levels of magnetite in various biological and laboratory materials[10,11]. It rapidly became clear that unique clean-laboratory techniques were required for this work because of the ubiquitous presence of ferromagnetic contamination. This contamination included ferromagnetic particulates present not only in the dust in the air, but also adsorbed onto the surfaces of laboratory equipment, present within glass and plastics, and even in reagent-grade laboratory chemicals and water.

We have encountered the same problem in our recent attempts to grow cells in tissue culture for an investigation of their magnetic properties. It is customary to use disposable, pre-sterilized plastic labware (flasks, pipettes, centrifuge tubes, and so on) and commercially prepared culture media in tissue-culture experiments because of their convenience and the assumption of a high level of quality control and cleanliness. We have found that none of these materials is free of ferromagnetic particulate contamination. Liquid-transfer manipulations, typical of cell-culture protocols, wash these particles from the surfaces of flasks and pipettes, and concentrate them with the cells during centrifugation. As an example, in a sham experiment we used 50 ml of leukocyte culture medium to rinse ten plastic T-250 flasks, ten 10-ml pipettes and ten 50-ml centrifuge tubes. After final centrifugation, we detected the equivalent of 160 ng magnetite in the rinsate, and the magnetic data indicated that the contaminants are small particles, usually in the sub-100-nm size range. As 160 ng magnetite equates to about 32 million 100-nm^3, this can be compared to the approximately 1 million cells that would have been produced in an equivalent culture volume.

Magnetite particles, 100 nm in diameter, either naked or coated with bovine serum albumin, are readily taken up by human white blood cells, including non-phagocytic lymphocytes as well as phagocytes[12]. Because the ferromagnetic particles interact strongly with magnetic fields, their presence in cell cultures, at a number density far higher than that of the cells, may provide a simple mechanism to account for links between EMF exposure and *in vitro* biological effects. A simple calculation shows that the mechanical energy present in a single 100-nm magnetite crystal exposed to a 60-Hz, 0.1-mT magnetic field is many times the thermal background noise[13]. Such particles, if adsorbed on cell surfaces or ingested by the cells, could conceivably transfer this energy to contiguous cell structures such as mechanically activated ion channels (which operate with a gating force close to the thermal noise limit[14,15]), and thereby alter cytoplasmic ion concentrations sufficiently to produce the observed biological effects.

We are not aware that the authors of any of the published studies of *in vitro* EMF effects have either controlled for, or attempted to reduce the levels of, ferromagnetic contamination. Although this is understandable, because the particles are difficult to detect and quantify except by sensitive magnetometry, their existence should not be ignored. *In vitro* studies may ultimately provide the information that will explain the connection between EMF exposure and biological effects, and as such they constitute roughly half of the projects at present being sponsored by the 5-year, $65 million NIEHS/DOE research programme on the biological effects of EMF. However, any effect of EMF exposure on cultured cells, if it is due to the presence of ferromagnetic contami nants, would have no relevance to *in vivo* biology. Data used to establish human exposure standards to electromagnetic fields must rely on properly controlled experiments.

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Extant fauna of ancient carbon

SIR — We have found that organisms of the rich, freshwater biological community involving sponges, flatworms and other benthic species near a thermal vent at the bottom of Lake Baikal are built of ancient carbon lacking ^{14}C.

The vent occurs at a depth of 420 m in Frolikha Bay (55°31′ N, 109°46′ E)[1,2]. It was found earlier that the carbon of its benthic organisms was produced by methanogenic bacteria, as revealed by the very small values of $\delta^{13}C$ (-60 to $-72‰$)[2]. However, the age of this carbon was not known.

Using a Tandetron accelerator mass-spectrometer, we measured the contents of ^{14}C in carbon of two *Bdellocephala* flatworms and a sponge collected on a bacterial mat of Frolikha vent, and found them to be equal to 0.43, 0.34 and 0.28 of that typical of modern organic matter, corresponding to apparent radiocarbon ages of 6,860 ± 260, 8,740 ± 80, and 10,200 ± 220 years before present (BP), respectively.

Hence, about 60–70 % of the carbon of the near-vent organisms has originated from ancient methane, rather than from modern atmospheric CO_2 due to photosynthesis or methanogenesis. The source of ancient carbon was also not limestone, as is sometimes the case in freshwater systems, since the uppermost layer of Baikal sediments is known to have an apparent radiocarbon age of less than 1,000 years BP at many locations (see ref. 3 and T. N. *et al.*, unpublished data).

Frolikha vent arises from meteoric water seeping through Baikal sediments[4] that are known to contain high concentra-

tions of methane; gas hydrates are ubiquitous under the floor of the lake, as shown by seismic profiling[5]. The small dependence of the near-vent community on photosynthesis suggests that vents of this kind could have been important in the nascence of the unique faunistic complex of Lake Baikal consisting of 1,500 endemic species: vents could have many times served as refuges under unfavourable climates, and sources of species radiation under more favourable ones during the 20-million-year-long history of the lake. Communities of organisms built of ancient carbon are not uncommon in a marine ecosystem[6], but this is the first time they have been found in a freshwater ecosystem.

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Another *obese* gene function

SIR — The cloning of the *obese (ob)* gene[1] is indeed a breakthrough in obesity research. As Rink highlighted in News and Views[2], the evidence strongly suggests that the normal *ob* protein is a previously undescribed hormone which regulates satiety. I would like to comment on another potential function of the *ob* gene.

When allowed free access to food, *ob/ob* mice eat more than normal *ob/–* mice and develop obesity. However, the lean body mass of *ob/ob* mice is lower than *ob/–* mice, and shows characteristics of stunted animals[3]. Furthermore, food restriction to the level of the normal *ob/–* mouse does not reduce adiposity of the *ob/ob* mouse, but it only causes a further decrease in lean body mass with little change in fat/lean body ratio compared withthe *ob/ob* mouse fed without food restriction[4]. The same results are observed in the *fa/fa* rat, another genetic model of obesity caused by a single recessive gene[5]. The *fa/fa* rat is thought to have a homologous defect to the *db/db* mouse[6], which may have a defective receptor for the *ob* protein[2].

These effects of the *ob* gene cannot be explained by suppression of appetite and subsequent reduction of food intake. Rather, the observations indicate that the *ob* protein regulates the energy partition between fat deposits and the lean body by some mechanism(s) not secondary to the effect on food intake, and that a defect in *ob* gene function causes an increased energy flow toward fat accumulation even at the expense of lean body growth.

In humans, uncommon obesity caused by a single gene has been reported. On the other hand, humans generally show increased lean body mass when the degree of obesity increases; and the treatment of obesity by energy restriction effectively reduces body fat without reducing lean body mass. Thus, it will be particularly interesting to determine the role of the human homologue of the mouse *ob* gene in human obesity. The discovery of the *ob* gene sequence will soon lead to the answer to this question. Even if this gene has little relevance to most obesity in humans, the potential use of the *ob* protein for the treatment of obesity will remain.

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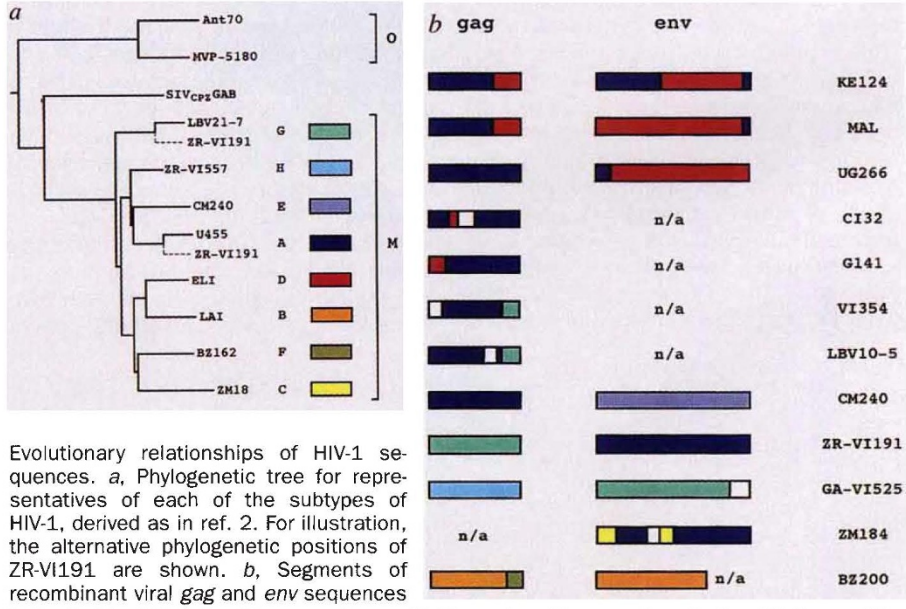
Recombination in HIV-1

SIR — Globally circulating strains of human immunodeficiency virus type 1 (HIV-1) exhibit extreme genetic diversity[1–5]. Phylogenetic analyses (*a* in the figure) have revealed two distinct 'groups' (M and O), and numerous 'sequence subtypes' within the major group M. Even though retroviruses (such as HIV-1) are highly recombinogenic[6], recombination among viruses from different subtypes has not been considered to be a significant source of new variation in HIV-1 because evidence for coinfection with multiple divergent HIV-1 strains has remained rare[7]. Here we report an extensive analysis of published HIV-1 sequences which reveals a surprisingly large number of apparently recombinant viruses. This finding has immediate consequences for our understanding of HIV-1 pathogenesis and for vaccine development[8], and of course implies that coinfection with divergent HIV-1 strains is not as rare as previously thought.

Recombination can be detected when different genes, or different regions within the same gene, are placed by phylogenetic analysis into different sequence subtypes. A single HIV-1 isolate (MAL) has long been suspected to be recombinant, and we have recently described[9] a detailed analysis of this viral genome in which the crossover points were localized. Here we have applied the same techniques to examine all HIV-1 isolates for which near-full-length *gag* or *env* sequences have been



Evolutionary relationships of HIV-1 sequences. *a*, Phylogenetic tree for representatives of each of the subtypes of HIV-1, derived as in ref. 2. For illustration, the alternative phylogenetic positions of ZR-VI191 are shown. *b*, Segments of recombinant viral *gag* and *env* sequences belonging to different subtypes; colour coding is as in *a*. Some segments (in white) cannot be designated as belonging to any currently known subtype. Localization of the breakpoints between regions of differing phylogenetic affinity is described in the table.