

subjects with two APOE- $\epsilon 4$ alleles, 3.1 years in subjects with one APOE- $\epsilon 4$ allele, and 0.6 years in subjects who did not have APOE- $\epsilon 4$ alleles. As subjects with earlier onset and longer duration are more likely to be diagnosed, we suspect that most diagnoses of AD and most prevalent cases are in subjects with one or two copies of APOE- $\epsilon 4$.

It is important to realize that 19 of 95 affected subjects in our cohort of pedigrees and 64 of 176 autopsy-confirmed sporadic AD cases described by Saunders *et al.* (6) had no copies of APOE- $\epsilon 4$. Twelve of 42 late onset families had affected members who did not have APOE- $\epsilon 4$. The fact that these tended to be the largest and, based on simulation studies (16), the potentially most informative families for linkage, suggests that other genetic sources of risk exist. These other genes will only be identified once the effects of APOE- $\epsilon 4$ are included in subsequent analysis. Inclusion of APOE- $\epsilon 4$ as a covariate in ongoing genomic screens will be necessary.

There are two possible mechanisms for generating the allelic association that we observed. The first is through genetic linkage disequilibrium to the actual risk-causing mutation. Genetic linkage disequilibrium arises when two loci are so close together that recombination very rarely occurs. Thus specific alleles may be passed through many generations in *cis* orientation, leading to an increase in the linked *cis* allele, despite the fact that it has no biological role in increasing risk. The previous report suggesting genetic linkage of late onset AD to loci residing near APOE (5), and the suggestive evidence for genetic linkage of the APOE locus to AD could support this explanation.

However, biological association, where the risk allele has an actual pathogenetic role, can mimic genetic linkage (17), and would result in similar positive genetic linkage results. In addition, the strong and compelling allelic dose effects are unusual, even in diseases with well-characterized associations (18, 19). Such dose effects are difficult to explain through genetic linkage.

Although the mechanism by which APOE- $\epsilon 4$ participates in pathogenesis is not known, the protein encoded by APOE- $\epsilon 4$ (apoE isoform 4) is immunoreactive in the plaques and neurofibrillary tangles that define the phenotype (20, 21), apoE isoform 4 has higher avidity *in vitro* for β -amyloid than the apoE isoform 3 (22) and subjects with two APOE- $\epsilon 4$ alleles exhibit greater β -amyloid staining at autopsy than other AD patients (23). The data, in conjunction with the statistical association, support the direct involvement of APOE- $\epsilon 4$ in the pathogenesis of AD.

We should caution that accurate application of these results to the general population, especially the 3% who are homozygous for APOE- $\epsilon 4$, will require population-based epidemiologic studies. However, in this sample of 42 families, the APOE- $\epsilon 4$ allele in a double dose was nearly sufficient to cause AD by age 80, and suggests that APOE- $\epsilon 4$ gene dose is a major risk factor for late onset AD.

REFERENCES AND NOTES

1. P. H. St George-Hyslop *et al.*, *Science* **235**, 885 (1987).
2. G. D. Schellenberg *et al.*, *ibid.* **258**, 668 (1992).
3. P. H. St George-Hyslop *et al.*, *Nature Genet.* **2**, 330 (1992).

4. A. Goate *et al.*, *Nature* **349**, 704 (1991).
5. M. A. Pericak-Vance *et al.*, *Am. J. Hum. Genet.* **48**, 1034 (1991).
6. A. M. Saunders *et al.*, *Neurology*, in press.
7. W. J. Strittmatter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1977 (1993).
8. M. A. Pericak-Vance *et al.*, *Exp. Neurol.* **102**, 271 (1988).
9. G. G. Koch and P. B. Imrey, *Analysis of Categorical Data* (Les Presses de L'Universite de Montreal, Montreal, 1985).
10. SAS Institute, Inc., SAS/STAT User's Guide, Release 6.03 Edition (SAS Institute, Cary NC, 1988).
11. D. R. Cox and D. Oakes, *Analysis of Survival Data* (Chapman & Hall, London, 1984).
12. SAS Institute Inc., SAS Technical Report P-217, SAS/STAT Software: The PHREG Procedure, Version 6 (SAS Institute Inc., Cary NC, 1991).
13. L. Wei, D. Y. Lin, L. Weissfeld, *J. Am. Stat. Assoc.* **84**, 1065 (1989).
14. R. G. Miller, Jr., *Survival Analysis* (Wiley, New York, 1981).
15. SAS Institute Inc., *SAS User's Guide: Statistics, Version 5 Edition* (SAS Institute Inc., Cary NC, 1985).
16. E. H. Corder *et al.*, in preparation.
17. D. A. Greenberg, *Am. J. Hum. Genet.* **52**, 135 (1993).
18. M. Langdon *et al.*, *Lancet* **ii**, 1178 (1984).
19. N. R. Farid *et al.*, *J. Clin. Invest.* **63**, 108 (1979).
20. Y. Namba *et al.*, *Brain Res.* **541**, 163 (1991).
21. T. Wisniewski and B. Frangione, *Neurosci. Lett.* **135**, 235 (1992).
22. W. J. Strittmatter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* in press.
23. D. E. Schmechel *et al.*, *ibid.*, in press.
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TECHNICAL COMMENTS

Composition Limits of Fe_xO and the Earth's Lower Mantle

The report by C. McCammon (1) addresses a significant problem in planetary geochemistry and should motivate a new generation of experimental and theoretical studies on magnesiowüstite. She concludes that pressure reduces the amount of Fe³⁺ in Fe_xO wüstite equilibrated with Fe (1). Although we would like to agree, her results do not demonstrate this, nor do they support her broader inference that the Earth's lower mantle is out of equilibrium with metallic Fe (for example, that of the core).

An essential difficulty in studying wüstite, a phase of considerable interest in the

materials and geological sciences, is that it is invariably nonstoichiometric ($x \neq 1.0$), with poorly characterized deficiencies (vacancies) at both oxygen and Fe sites and variable amounts of Fe²⁺ and Fe³⁺ in crystalline and interstitial-defect sites (1). Therefore, at least three compositional variables are required to describe wüstite, and it is impossible to associate uniquely the deviation from stoichiometry with the use of an Fe³⁺/Fe²⁺ ratio, as McCammon seems to assume. In addition, there is evidence that the stoichiometry varies as a function of composition across the

(Mg,Fe)O magnesiowüstite series that is relevant to the Earth's lower mantle.

The correlation between stoichiometry and lattice parameter assumed by McCammon and her co-workers (1, 3) depends on synthesis conditions and is uncalibrated (indeed, unverified) for wüstites synthesized at elevated pressures (2). Wüstite exhibits an irreversible change in lattice parameter after hydrostatic compression above 12 to 15 GPa at room temperature (4); presumably this phenomenon reflects easy changes in defect structures and is unrelated to stoichiometry. McCammon's report illustrates the significance of wüstite in constraining the Earth's geochemical evolution and thus highlights the need to calibrate simple methods of determining wüstite compositions at high pressures and temperatures.

The new data (1) neither prove nor disprove the earlier contention (3) that x decreases below 0.98 at lower mantle pres-

sures (above 20 to 25 GPa). McCammon's call for in situ electrical conductivity measurements has been answered through shock-wave and diamond cell experiments which show that wüstite undergoes a fundamental bonding change at deep mantle conditions: it becomes metallic at approximately 70 GPa, far beyond the range of McCammon's experiments, yet well below the pressures of the boundary between the core and the mantle (5). The change in chemical bonding has profound implications for inner Earth dynamics and invalidates conclusions about metal oxide equilibration deep in the lower mantle that are based exclusively on experiments at pressures below 30 to 40 GPa (5, 6).

Finally, geophysical measurements of deep mantle electrical conductivity do not require the presence of Fe³⁺, because either increased Fe²⁺ or hydrogen contents influence the conductivity in the manner required to match the observations (7). Therefore, the state of equilibration between the mantle and core remains essentially unconstrained.

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REFERENCES

1. C. McCammon, *Science* **259**, 66 (1993).
2. R. M. Hazen and R. Jeanloz, *Rev. Geophys. Space Phys.* **22**, 37 (1984).
3. C. A. McCammon and L. Liu, *Phys. Chem. Miner.* **10**, 106 (1984).
4. R. M. Hazen, H. K. Mao, L. W. Finger, P. M. Bell, *Carnegie Inst. Washington Year Book* **80**, 274 (1981); R. Jeanloz and R. M. Hazen, *Nature* **304**, 620 (1983).
5. E. Knittle, R. Jeanloz, A. C. Mitchell, W. J. Nellis, *Solid State Commun.* **59**, 513 (1986); E. Knittle and R. Jeanloz, *J. Geophys. Res.* **96**, 16169 (1991).
6. E. Knittle and R. Jeanloz, *Geophys. Res. Lett.* **16**, 609 (1989); *Science* **251**, 1438 (1991); F. Goarant, F. Guyot, J. Peyronneau, J. P. Poirier, *J. Geophys. Res.* **97**, 4477 (1992).
7. X. Li and R. Jeanloz, *J. Geophys. Res.* **96**, 6113 (1991); *Nature* **350**, 332 (1991).

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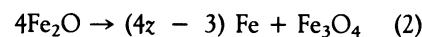
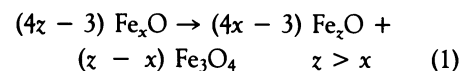
Response: My experiments, which used a multianvil press, showed that the Fe content of Fe_xO increased with pressure. These data and several assumptions led me to the inference that the Earth's present lower mantle is not in equilibrium with Fe (1). Jeanloz and Hazen, however, challenge the experimental observation that pressure increases the Fe content of Fe_xO and hold that the state of equilibration between the

mantle and core remains unconstrained.

My experimental results were obtained by synthesizing Fe_xO samples at high pressure and temperature and then quenching them to ambient conditions. The Fe composition was obtained from the linear correlation between cubic cell parameter and iron composition and from Fe³⁺ fraction as determined by Mössbauer spectroscopy. Results from the latter analysis directly refute the statement by Jeanloz and Hazen that my experiments do not demonstrate a reduction in the Fe³⁺ content of Fe_xO at high pressure. The Mössbauer spectra of all samples were recorded both above and below the Néel transition, and in both types of spectra a decrease in ferric content with increasing pressure was evident. The Fe³⁺ fractions determined from quantitative fits to the Mössbauer data are consistent with the composition determined from the cell parameter.

Jeanloz and Hazen state that the correlation between Fe content and lattice parameter depends on synthesis conditions, which leads to an error of up to 0.02 in Fe content (2). This statement is based on the experimental data of Carel and Gavarri (3) and the literature compilation of Bauer and Pianelli (4). Carel and Gavarri performed two experiments with quenched samples: They studied the variation in lattice parameter (i) at constant composition for different equilibrating temperatures and (ii) at constant equilibrating temperature for different compositions. The first experiment showed a difference in lattice parameter

corresponding to an uncertainty in Fe content of only 0.001 for samples quenched between 890° and 1340°C (5). The second experiment showed that a systematic shift with equilibration temperature, if present, is smaller than the experimental uncertainty (Fig. 1). The results of my synthesis experiments (Fig. 1) are in agreement with the published lattice parameter/Fe content correlation (6). The scattered pattern of the data, noted by Bauer and Pianelli (4), is almost certainly a result of quenching effects. Fe_xO is unstable below 570°C, and disproportionation occurs according to the following process (7).



There is sufficient kinetic energy to drive reaction 1 forward during quenching, and although the total composition remains constant during the reaction, the cell parameter of Fe_xO increases as a result of the higher Fe content. A recent x-ray diffraction study has confirmed the presence of two distinct compositions of Fe_xO during cooling from high temperature, where the relative proportion of the more Fe-rich composition increases with increasing cooling time (8). Data from Bauer and Pianelli's compilation fall into two categories: those that agree with the lattice parameter-Fe content linear correlation and those that have lattice parameters that are too large for a given Fe composition. My samples were quenched in ice water, which appears to have prevented significant transformation during quenching (Fig. 1). Samples in the multianvil press were quenched from high temperature while pressure was held constant; because the stability field of Fe-rich Fe_xO expands at high pressure (1), disproportionation effects were most likely minimized.

Although a metallic transition has been observed in Fe_xO at approximately 70 GPa (9), I know of no evidence that (Fe,Mg)O undergoes such a transition within the pressure range of the Earth's lower mantle, and electrical conductivity studies of (Fe,Mg)O with up to 27.5% FeO show no evidence of metallic behavior at 30 GPa and 2000 K (10).

Although geophysical measurements of lower mantle conductivity are not conclusive of the presence of Fe³⁺ in lower mantle magnesio-wüstite, the alternative possibilities seem at least as speculative. There is no conclusive evidence that the lower mantle is enriched in Fe relative to the upper mantle, and it is not clear that hydrous silicate assemblages would be stable in the lower mantle at conditions of metal equilibrium. Nevertheless, assumptions about

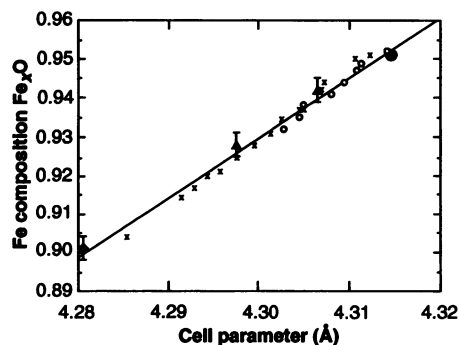


Fig. 1. Variation of lattice parameter with iron composition for Fe_xO quenched from the following temperatures: 900°C (solid triangles); 1000°C (solid circle); and 1200°C (solid diamond). The lattice parameter was determined from x-ray diffraction cell refinement data, and the Fe compositions were calculated from either the CO/CO₂ gas ratios (900° and 1200°C data) or the composition of the Fe-Fe_xO phase boundary (1000°C) (11). Errors in content determination are estimated to be ±0.003 and ±0.001, respectively. The straight line represents the linear relation used to determine Fe compositions (1). Also plotted are the data of Carel and Gavarri (4) at 1000°C (open circles) and 1200°C (crosses).