the total cross section for the species to the total cross section of the entire atmosphere does. "Total cross section" refers to the product of the number density and the actual cross section (consider the trivial case of a mono-species atmosphere where changing the cross section of the species has no effect because only that species can be sputtered). At the altitudes of interest, CO_2 is the dominant (75%) constituent. Decreasing its cross section will also decrease significantly that of the entire atmosphere, and the net effect of the decrease will be much smaller. For channel I, the effective decrease is only about a factor of 2; for channel II, it is about a factor of 4.

The effect of changing the cutoff energy for dissociation does not linearly affect the collision frequency. With regard to the results of our model, 85% of the O in collisions that are above the dissociation threshold stated in our report (*1*) are above that of channel I, and 40% are above that of channel II. In the affected collisions, most of the energy goes into breaking the molecule, so almost none of the particles from such collisions have sufficient energy to escape, further reducing the effects of changing the cutoff energies. Our revised model indicates that the higher cutoff energies have no effect in channel I and results in a factor of two decrease in yield for channel II.

While channel I does not produce C directly, it is still relevant to the process. First, the mass of CO is less than a third that of CO_2. It thus has a significantly lower escape energy, and even partially dissociating the CO_2 increases the sputtering yield. Second, the mean number of collisions that an escaping CO_2 fragment undergoes is about four (and over 75% undergo at least two collisions). Thus, while the CO_2 will not be completely dissociated in its first collision, subsequent ones will finish it. The C will end up escaping in elemental form as our original model assumed, giving it the significant mass advantage that accounted for so much of the difference between our results and those made earlier (*2*, *3*). This advantage allows channel I (which does not directly produce C) to contribute significantly to the final sputtering yield. These two effects allow channel I to be less efficient than complete dissociation by only a factor of 2.

As in our report, we used the impacting ion flux used by Luhmann (*2*) and Jakosky (*3*). There is a feedback between the escaping flux and the impacting flux, but this does not affect the actual sputtering yields. Calculated total CO_2 loss is affected, but there are other, larger uncertainties in the impacting flux. There are two typographical errors in table 2 of our report (*1*). The Exospheric O value at 1 EUV should read 8×10^{25} and the Escaped H_2O at 6 EUV should read 1.9×10^{28}. Neither error affects the calculations or figure 1 of our report (which were done with the correct values).

With both channels contributing to the sputtering of C (either as C or as CO), our revised model implies a loss of about 1 bar of CO_2 (not ~3 bar, as we originally stated). This decrease can be attributed to the decrease in effective yield of channel I by a factor of four and that of channel II by a factor of eight relative to the cross section stated in our report (*1*). This is only a small part of the total difference between our results and those of Luhmann (*2*) and Jakosky (*3*). One bar of CO_2 is the canonical amount needed by greenhouse models to create the warmer, wetter early Martian atmosphere (*4*). Our revised results imply that, because of dissociation during collisions, sputtering is an important loss mechanism.

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Table 1. Effects of the revised cross sections. First column is the actual change between the cross section presented in our report (*1*) and in the comment by Johnson and Liu; second is the effect that Johnson and Liu state that this change will have on the number of collisions (and thus the sputtering yield); third is the effect that we attribute based on statistics from our model. Plateau is the value over the "flat" portion of the cross section. Cutoff is the minimum energy required for the collision to occur. Inefficiency is the reduction in effect in channel I because it does not directly produce C. Values are all expressed as multiplicative factors. In all cases, the final values are smaller than those calculated earlier. Not calculated, –.

	Cross section change	Stated effect (J&L)	Modeled effect (K&Y)
Plateau			
Channel I	5	5	2
Channel II	10	10	4
Cutoff			
Channel I	1.2	–	1
Channel II	5	5	2
Inefficiency			
Channel I		∞	2
Total			
Channel I	6	–	4
Channel II	50	50	8
Combined	5	50	3

Alzheimer's Precursor Protein and the Use of Bathocuproine for Determining Reduction of Copper(II)

Gerd Multhaup *et al.* (*1*) report the ability of amyloid precursor protein (APP) to reduce Cu(II) to Cu(I) as determined through use of a specific colorimetric ligand for Cu(I), bathocuproine disulfonate (BC). Their experimental protocol duplicated a previous use of BC to determine the ability of low density lipoprotein (LDL) to reduce Cu(II) (*2*). Both groups stated that BC could be used to quantitate Cu(I) because of its lack of interaction with Cu(II). However, the references they cited to support this statement do not make such an assertion. In fact, not only is it well known that BC binds to Cu(II) to give the bis complex $(BC)_2Cu(II)$, but BC thereby also makes Cu(II) a nearly 0.5-V stronger oxidizing agent than usual (*3*). This results from the 2,9-dimethyl substitution on the phenanthroline ligand sterically preventing planar tetracoordination and forcing instead a Cu(I)-preferring tetrahedral-like coordination on Cu(II). In the presence of BC, Cu(II) has been observed to oxidize "biological" buffers such as HEPES (*4*) and PIPES (*5*), a reaction which we demonstrated to reflect oxidation of the tertiary amine center in these buffers (*6*). The main purpose of this comment is to call attention to the fact that BC is not an innocent indicator of Cu(I) and that any protein oxidation observed in using it could well represent a methodologic artifact.

On the basis of the expected stability constants, one can calculate that at the concentrations of Cu(II) and excess BC used in the above two studies (*1*, *2*), 99.5% of the Cu(II) will exist as $(BC)_2Cu(II)$ (*7*) before addition of protein. Thus, in these studies, APP and LDL are being exposed to a predominantly highly oxidizing, nonphysiologic form of Cu(II). Certain Cu(II)-coordinating ligands and protein sites that are capable of competing with BC for binding to Cu(II) can displace one or both BC ligands, thereby abrogating the unusual oxidizing strength of Cu(II). This explains why certain added ligands such as Gly-His-Lys inhibit reduction of Cu(II) by APP (*1*) and why certain proteins that bind Cu(II) strongly may not show any reduction of Cu(II) even though they contain moieties that normally reduce $(BC)_2Cu(II)$.

In the case of APP, Multhaup *et al.* provide evidence that Cys^{144} in APP is involved in the reduction of Cu(II)/BC (*1*). In the absence of BC, it is possible that this same Cys oxidation could occur according to a Cu(II)-catalyzed autoxidation process. If so, this reaction could generate the reactive oxygen species (ROS) discussed by Multhaup *et al.* (*1*), though the quantity of ROS would be limited to the small, stoichiometric amounts of Cys-containing protein present, in contrast to the larger amounts of ROS characteristic of cytotoxic redox cycling phenomena. In this regard, one should keep in mind that a Cu(II)-catalyzed autoxidation differs from stoichiometric reduction of Cu(II) in the presence of BC, especially in that $(BC)_2Cu(I)$ is inert to O_2-mediated reoxidation. The conclusion by Multhaup *et al.* (*1*) that neither superoxide nor H_2O_2 is involved in the reduction of Cu(II) by APP, as judged by the lack of effect of added superoxide dismutase or catalase, should not be a general interpretation—superoxide and H_2O_2 are not expected to interfere with the $(BC)_2Cu(II)$ reaction, but they could well alter the status of APP-bound copper in the absence of BC.

Also, the observation that Fe(III) is not reduced by either APP (*1*) or LDL (*2*) might appear contradictory based on the fact that the indicator ligand used in this case, bathophenanthroline disulfonate (BP), binds to Fe(III) as well as to Fe(II) and makes the potential even higher (*8*) than for $(BC)_2Cu(II)$. The lack of observed reduction of Fe(III) reflects the fact that the octahedral tris complex formed in this case, $(BP)_3Fe(III)$, is coordinatively saturated and thus kinetically incompetent in thiol oxidation. This is in contrast to $(BC)_2Cu(II)$, which effects rapid inner-sphere oxidations via pentacoordination (*6*, *7*).

In summary, batho-based ligands cannot be used to monitor reduction of Cu(II) and Fe(III) that occurs physiologically. In fact, there is at present no easy way to do this because the reduced metals would normally be reoxidized by O_2, and any indicator ligand that prevents this would concomitantly alter the iron/copper redox properties. In the cases mentioned above, one cannot thus conclude definitively that either APP or LDL is capable of spontaneous physiologic reduction of Cu(II). The same concern applies to a recent report that α-tocopherol acts as a prooxidant in human lipoproteins by reducing Cu(II) to Cu(I) (*9*); such action probably reflects merely the inclusion of BC to monitor the Cu(I) formed.

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Response: We summarize our evidence for the reduction of copper(II) to copper(I) by APP. First, as shown previously by us (*1*), APP has a high affinity binding site for copper(II), which is located within residues 135 to 155 of APP. This binding site is conserved in the related protein APLP2.

Second, complex formation between copper(II) and a synthetic peptide representing this copper(II) site in the absence of bathocuproine resulted in cysteine oxidation. The oxidized peptide still binds copper as shown by LC-ESI-MS. Recent electron paramagnetic resonsance (EPR) analysis (*2*) showed that the cysteine oxidation is accompanied by the disappearance of the copper(II) signal.

Third, EPR analysis revealed also that copper(II) was not reduced when bound to a peptide representing the copper binding site of APP in which only cysteine was replaced by serine. This shows the importance of cysteine in copper(II) reduction by the APP peptide and that no other reducing agents are required (such as molecular oxygen, possibly present in the buffer).

Fourth, our experiments performed with bathocuproine to measure copper(I) formation showed within seconds the characteristic change of absorbance at 480 nm. Such a rapid change has never been found by us when we incubated copper(II)-bathocuproine complexes in the reaction buffer, without APP or its copper-binding site peptide, even after overnight incubation.

In conclusion, our finding of an enzyme-like activity of APP in the reduction of copper(II) to copper(I) is not solely based on bathocuproine data and does not depend on copper(II)-bathocuproine complex formation.

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than 11,000 years B.P. from Clovis sites was analyzed for total collagen, considered an unreliable material, or have extremely large errors (of 450 to 600 years). The oldest, from Domebo, Oklahoma, were saturated with creek water draining a petroleum oil field [F. C. Leonhardy, *Domebo: A Paleoindian Mammoth Kill in the Prairie Plains* (Contributions of the Museum of the Great Plains, No. 1, Lawton, OK, 1965), pp. 3–9 and 14–26.] The dates for amino acid suites of well-preserved bones have smaller errors and postdate 11,000 years B.P. The Amazonian dates, in contrast, were for large samples of taxonomically identified, spatially plotted seeds of fruits used by humans for food. Seeds are short-lived plant parts and thus lack inherent age, and we tested them (p. 380) for contamination by blind tests of split samples of solids and solutes.

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9. A. Prous writes [*J. Soc. Am.* **77**, 77 (1991)], "The climate is hot, almost semiarid, with . . . precipitation around 700 mm a year." In contrast, the rainfall in a 50-kilometer radius around Monte Alegre ranges from 2000 to 3000 millemeters a year, more than three to four times that at Boquete.

Letters to the Editor

Letters may be submitted by e-mail (at science_letters@aaas.org), fax (202-789-4669), or regular mail (*Science*, 1200 New York Avenue, NW, Washington, DC 20005, USA). Letters are not routinely acknowledged. Full addresses, signatures, and daytime phone numbers should be included. Letters should be brief (300 words or less) and may be edited for reasons of clarity or space. They may appear in print and/or on the World Wide Web. Letter writers are not consulted before publication.