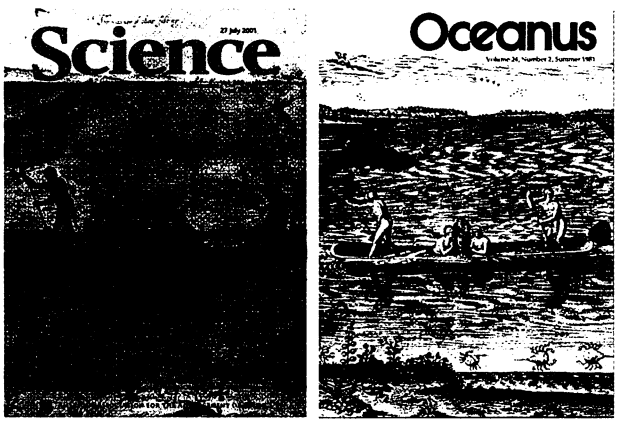
Mixed Messages from the Distant Past?

THE STRIKING 16TH-CENTURY COVER illustration of the Algonquian fishermen on Pamlico Sound (special issue on Ecology Through Time, 27 Jul.) looked vaguely familiar. Casting about, I soon found the Summer 1981 issue of *Oceanus*. *Science*'s illustration, credited to John White (1585), is so similar to that on the *Oceanus* cover, credited to Theodor de Bry (1590), that there can be little doubt that one is parent to the other (see the figure). If we accept the dates, then de Bry has embellished and possibly corrected White's portrayal of these early fishing practices.

The oddly constructed holding pen in White's illustration has become an elaborate (and more likely) fish trap. Both versions of the scene teem with sea life, but de Bry's much the more so, in seeming contradistinction to the fact that he also has added considerably to the fishing effort. The fish traps line the distant shore in de Bry's version, and many more canoes and spear fishers dot the scene.

The point made in *Science*'s cover caption is that the vision of natural abundance revealed in this long-ago moment in time stands in contrast to contemporary perspectives in which humans are an increasingly dominant and worrisome element. But perhaps de Bry's embellishments hold a deeper message as to why overfishing is the powerful agent of ecological extinction that it has become (*1*). The more we perceive our fellow humans to be benefiting from freely available natural abundance, the more we want to believe that the world is constructed so as to invite us to do it too—in short, Hardin's "tragedy of the commons" (*2*). To my eye, therefore, the real message of both illustrations is allegorical, embodied in the most obvious element they have in common—the curious image, dead center, of two humans seemingly intent upon burning a hole directly through the bottom of their canoe.



Cover comparison. The *Science* cover from 27 July (left) and the *Oceanus* cover from Summer 1981 (right).

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted by e-mail (science_letters@aaas.org), the Web (www.letter2science.org), or regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

The Two Faces of Vitamin C

S. H. LEE AND CO-AUTHORS SUGGEST ON THE basis of their research findings that high doses of vitamin C could potentially promote DNA damage that could lead to cancer (Reports, "Vitamin C–induced decomposition of lipid hydroperoxides to endogenous genotoxins," 15 Jun., p. 2083). Their report could leave the impression that no human studies have been performed to address this question.

In fact, five human studies have been conducted that do not confirm Lee *et al.*'s speculation (*1*–*5*). For example, researchers at Johns Hopkins University could not find evidence of a "significant main effect or interaction effect on oxidative DNA damage in non-smoking adults" with 500 mg per day of vitamin C supplementation (*1*). In a German study, researchers found that 1000 mg of vitamin C consumed by smokers and nonsmokers for 7 days did not produce DNA damage, as measured by the number of micronuclei in blood lymphocytes (*2*). And in yet another study conducted by Immunosciences Laboratory in California, 20 healthy volunteers were divided into four groups and given either placebo or daily doses of 500, 1000, or 5000 mg of vitamin C (ascorbic acid) for 2 weeks. The researchers concluded that "ascorbic acid is an antioxidant and that doses up to 5000 mg neither induce mutagenic lesions nor have negative effects on natural killer cell activity, apoptosis, or cell cycle" (*3*).

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THE INDICTMENT OF VITAMIN C AS A POSSIBLE in vivo producer of genotoxins (molecules that damage DNA) by Lee and colleagues is based on a test-tube reaction that does not adequately represent the cellular environment. Cells have many ways of quenching free radical chains that include peroxidases, superoxide dismutases, and catalase, as well as other proton donors like glutathione and vitamin E, which can be maintained in the reduced state by vitamin C.

The authors cite a study showing that supplementary vitamin C (200 mg daily) can produce intracellular concentrations of 1.4 to 3.4 mm, implying that this level might be harmful. However, rats have about

6 mm vitamin C in their pulmonary alveolar fluid (*1*). This nearly twofold greater concentration apparently helps protect and maintain alveolar capillary membranes that mediate transport of O_2. A similarly high concentration of vitamin C is found in adrenal glands, where free radicals are routinely produced by the many hydroxylation reactions involved in the synthesis of epinephrine and steroid hormones.

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THE STUDY OF LEE AND COLLEAGUES demonstrates the decomposition of lipid hydroperoxides to form genotoxins in the presence of vitamin C and in the relative absence of transition metals. However, this in vitro reaction was carried out at a pH of 7.0, which is not physiologic and would represent severe acidosis in vivo. At a plasma pH of 7.0, which occurs with ischemic disease states, a large amount of weakly bound copper is released from ceruloplasmin and other proteins to oxidize low-density lipoproteins (*1*). The released free Cu(II) is then available to participate in Haber Weiss and Fenton reactions to generate reactive oxygen species and form genotoxins with resultant DNA damage (*2*).

"Lee *et al.* present no evidence for the role of vitamin C alone at physiological pH (7.4) in generating genotoxins."

This in vivo mechanism of DNA damage is probably more important than vitamin C alone. Lee *et al.* present no evidence for the role of vitamin C alone at physiological pH (7.4) in generating genotoxins. When acidosis is present, the Cu(II)–vitamin C interaction accounts for most of the DNA damage through the formation of radicals. Lee *et al.*'s suggestion that oral intake of vitamin C in physiological conditions (in the absence of acidosis) might have a damaging effect on DNA was not proven by their study.

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Responses

CONTRARY TO SARDI'S INTERPRETATION, WE did not suggest that high doses of vitamin C would cause DNA damage. We said in our report, "The efficiency of [vitamin] C in inducing the decomposition of lipid hydroperoxides suggests that this process could give rise to significant levels of DNA damage in vivo." Furthermore, we took great care to show that the effect we observed was not a pro-oxidant effect.

Four of the articles cited by Sardi (*1–4*) describe the formation of DNA adducts derived from oxidative damage and so are irrelevant to our findings. We actually referred to the article by Proteggente *et al.* (*3*) in our report as being illustrative of the potential for a transition metal ion–mediated pro-oxidant effect of vitamin C. As far as we are aware, nobody has examined the effects of vitamin C on lipid hydroperoxide–mediated DNA damage in humans. It is only recently that the methodology for such studies has become available (*5*).

The other study Sardi mentions, by Schneider *et al.* (*6*), was also primarily involved in measuring the consequences of oxidative damage to DNA. However, Schneider *et al.* did indeed show that there was no statistically significant effect of vitamin C on either lymphocyte sister chromatid exchange or the numbers of lymphocyte micronuclei in nonsmokers or smokers. Similarly, there was no significant difference in "TBARS," a crude index of lipid peroxidation products. This study did not monitor the formation of any lipid hydroperoxide–derived DNA adducts. Therefore, it is also not really relevant to our findings.

We accept that there is no evidence from short-term studies that vitamin C causes mutagenic lesions in healthy volunteers. However, the effects of vitamin C on different populations have not been studied in any detail. Since the submission of our report, we found out that there is evidence that vitamin C causes sister chromatid exchange in human lymphocytes in vitro (*7*). Furthermore, a paper published 2 weeks before our report showed that vitamin C enhances single-strand breakage induced by peroxynitrite in human myeloid leukemia cells (*8*). Peroxynitrite induces lipid peroxidation (*9*), so this makes it even more important to conduct long-term studies in different populations to determine whether lipid hydroperoxide–derived lesions in DNA can be detected.

Our study has provided a molecular target to study whether vitamin C (or other antioxidants) can induce lipid hydroperoxide–mediated DNA damage in vivo. Methodology is now available for the analysis of etheno-2′-deoxyadenosine (*5*), the lesion that we showed results from the reaction of 4,5-epoxy-2(*E*)-decenal with DNA. We anticipate that it will now be possible to test whether vitamin C can induce this mutagenic lesion (*10*) in different human populations.

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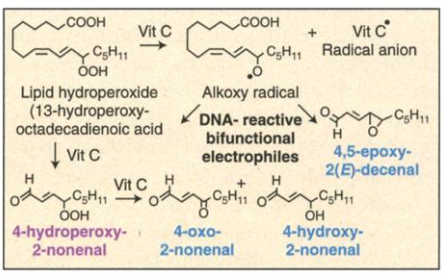
KOOBS IS CORRECT IN ASSUMING THAT vitamin C will prevent the formation of lipid hydroperoxides by scavenging free radical reactive oxygen species. However, lipid hydroperoxides can form by other pathways, such as those we suggested in our report (*1*). In this situation, vitamin C will cause the formation of DNA-reactive bifunctional electrophiles by inducing lipid hydroperoxide decomposition (see the figure). Intracellular concentrations of vitamin C in humans (*2*) are similar to those we used in our experiments. This is why we suggested that vitamin C could induce lipid hydroperoxide decomposition in vivo to the same DNA-reactive bifunctional electrophiles that we observed in our in vitro experiments. At concentrations of 6 mm vitamin C that Snyder *et al.* observed in rat pulmonary alveolar fluid (*3*), the same electrophiles would be formed if lipid hydroperoxides were also present.



Vitamin C–induced decomposition of lipid hydroperoxides to DNA-reactive bifunctional electrophiles.

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IN RESPONSE TO BAR-OR, WE ACCEPT the criticism that this study was conducted at pH 7.0 rather than pH 7.4. We were mainly concerned with eliminating transition metal ions and with conducting the reactions at neutral pH to show that we were not simply observing the well-known transition metal ion pro-oxidant effect (*1*). Clearly, the only way to prove that the lipid hydroperoxide–derived bifunctional electrophiles are formed at intracellular pH is to conduct both in vitro cell culture and in vivo experiments. From a theoretical perspective, there is no reason to suppose that a pH difference of 0.4 units would affect the reaction that we discovered. Thus, at pH 7.0 and pH 7.4, both vitamin C and polyunsaturated fatty acid hydroperoxides exist almost exclusively in their corresponding anion forms. Furthermore, small changes in pH have essentially no effect on the reactions of lipid hydroperoxide–derived bifunctional electrophiles with DNA. Below pH 6.0, 4,5-epoxy-2(*E*)decenal is unstable. However, this is well below the pH that we used in our reactions. Subsequent to the submission of our report, we tested the reaction of vitamin C with lipid hydroperoxides at pH 7.4 and found that the product profile was essentially the same as that observed at pH 7.0 (*2*).

"From a theoretical perspective, there is no reason to suppose that a pH difference of 0.4 units would affect the reaction that we discovered."

It is certainly conceivable that DNA damage caused by acidosis results from Cu/ascorbate-mediated radical formation rather than ascorbate alone. However, our study showed that lipid radicals could also be formed by the Cu/ascorbate system. This would result in generation of the same lipid hydroperoxide–derived bifunctional electrophiles that we reported. Furthermore, the mutagenic potential of the resulting DNA lesions, such as etheno-2′-deoxyadenosine (*3*), is much greater than that resulting from oxidative damage (*3*, *4*). This suggests that it will be important in the future to monitor human populations for DNA lesions that result from lipid hydroperoxide–derived genotoxins, as we suggested in our report.

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Sustainability: Insight from Industrial Ecology

SUSTAINABILITY IS ONE OF THE MOST important issues we face today, and thus it