*Response:* The model that we used in our report (*1*) is not incorrect. We would have arrived at the same conclusion regarding the seawater Sr isotopic consequences of Larson's crustal production rates during the mid-Cretaceous (*2*) had we considered in our report only the extrusive part of oceanic crustal production. This consideration would involve two changes to the model. The first would be to convert the total crustal production rate provided by Larson to the extrusive component by dividing the values by five (because, as Larson points out, the ratio of extrusive to intrusive is relatively constant at about 1 to 4, or 1 to 5). The second change would be to calculate the Sr exchanged per cubic kilometer of extrusive rocks. Given that the total amount of Sr exchanged is fixed at $1.2 \times 10^{10}$ mol per year (for 20 km³ of total crust or 4 km³ of extrusive component), the exchange per cubic kilometer of extrusives will be five times greater than that calculated for the oceanic crust as a whole. The combined effect of considering changes in the production rate of extrusives and attributing all Sr exchange to the extrusive part of the oceanic crust results in exactly the same calculated change in the Sr isotopic composition of seawater for the oceanic crustal production rates given by Larson (*2*). Thus it appears that the "incorrect use of crustal volume data" noted by Larson is irrelevant.

Larson suggests that a 28% increase in crustal production rates (not accounting for plateaus) during the mid-Cretaceous "is a close match to the largest increase calculated by Ingram *et al.* in hydrothermal flux." However, the value that we calculated based on our Sr data is 15% (*1*); not the 25% estimated by Larson from our figure 2. Following the equation given in our paper

(*1*, p. 549), one can calculate that a 28% change in the hydrothermal flux would produce a change of 40 $\Delta^{87}$Sr units while the actual data show a change of only half that amount. Furthermore, the 28% change in crustal production rate mentioned by Larson is an average for a period lasting some 40 million years, while the seawater Sr isotopic data indicate a period of low $^{87}$Sr/$^{86}$Sr lasting no more than 10 million years.

As summarized in our report (*1*), the mid-Cretaceous is an exceptional time in terms of oceanic volcanism, high sea level, high global temperature, and the preservation of large amounts of organic carbon, and yet the Sr isotopic composition of seawater, which is often assumed to be a useful monitor of global processes, shows little change. Further, we addressed the question of how much change in the Sr isotopic composition of seawater would result from the much larger oceanic crustal production rates suggested by Larson (*2*) under the assumption that new oceanic crust in the Cretaceous exchanges Sr in much the same way as does presently forming oceanic crust. All other factors in the Sr budget of seawater were for the purposes of this intellectual exercise held fixed. The result was that we calculated a decrease in the $^{87}$Sr/$^{86}$Sr of seawater that is five times larger in amplitude and five times longer in duration than what is observed. The only discernable effect was a decrease in $^{87}$Sr/$^{86}$Sr coincident with and proportional to the emplacement of the large oceanic plateaus. Perhaps changes in the hydrothermal Sr exchange from increased ocean crust production were compensated by almost exactly the right changes in the riverine flux of Sr. We noted that possibility in our report (*1*), but still believe it to be unlikely. Perhaps, as Larson points

out, it is a result of mid-Cretaceous oceanic volcanism having different Sr exchange properties than present-day oceanic volcanism. If that is the case, one should be especially cautious in using the high rate of oceanic volcanism during the mid-Cretaceous to explain other aspects of ocean chemistry during that period. Alternatively, perhaps the mid-Cretaceous ocean production rates are not entirely correct. Larson himself (*2*, p. 548) states "there are large assumptions in the calculation of Pacific ridge volume that probably never can be verified, but they must be utilized if such a worldwide calculation [of ridge production] is made." Thus it seems appropriate to use the Sr isotopic composition of seawater during the mid-Cretaceous to verify (or deny) some of these "large assumptions."

Larson mentions a paper by Jones *et al.* (*3*) that appeared after our report. Rather than respond to Larson's assertion that the paper by Jones *et al.* (*3*) produced a more "quantified and successful" model than ours, we suggest that interested persons read both and reach their own conclusions.

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# Mutant Mice, Cu,Zn Superoxide Dismutase, and Motor Neuron Degeneration

In their report of 17 June, Mark E. Gurney *et al.* (*1*) describe a transgenic mouse model for amyotrophic lateral sclerosis (ALS) that overexpresses a mutant human gene encoding Cu,Zn superoxide dismutase (SOD) and that also normally expresses mouse SOD, resulting in a fourfold increase in total SOD activity. A large literature shows that overexpression of SOD causes a paradoxical oxidative stress not unlike that associated with the *underexpression* of the gene. Gurney *et al.* do not cite this literature, however, and instead interpret their result to mean that familial ALS is not a result of the fact that these individuals have about 50% less SOD activity in their cells, but rather of

some new but unknown activity (a gain-of-function) coincidentally shared by the dozen or so mutant forms of the human SOD found so far in ALS patients.

Elroy-Stein *et al.* (*2*) noted substantially increased lipid peroxidation in transfected cells overproducing *native* human SOD by a factor of 3.6 and estimated that overexpression of SOD beyond a factor of 6 is probably lethal. Norris and Hornsby similarly concluded that overexpression of SOD is lethal to transfected adrenocortical cells (*3*).

We have reported that for any given rate of superoxide production there exists a concentration of SOD that will produce a minimum amount of oxidative stress and lipid

peroxidation. This is a result of the paradoxical abilities of the superoxide radical to both initiate and terminate lipid peroxidation (*4*). (Initiation is indirect, by the liberation and reduction of iron.) Thus, when exogenous SOD is used to restore oxidative balance to a tissue in oxidative stress, such as a postischemic isolated heart, it exhibits a relatively sharp bell-shaped dose-response curve (*5*). A unique concentration of the enzyme provides maximal protection; either more or less than this concentration leads to increased lipid peroxidation, increased biochemical markers of tissue damage, and loss of function.

The transgenic "ALS mouse" expresses four times more SOD activity than a normal mouse. The oxidative stress and increased lipid peroxidation resulting from this degree of overexpression would be substantially greater than that produced by ex-

pression of half the normal concentration of SOD, judging from the bell-shaped dose response curves (5). This would occur regardless of whether the activity were a result of a mutant form of SOD or of the native enzyme.

Why, then, does the ALS transgenic mouse develop symptoms of ALS when other mice transgenic for SOD do not develop these particular symptoms? Perhaps it is because the tissue distribution of an overexpressed transgene is variable and unpredictable, depending on where in the genome the transgene integrates. It may have nothing to do with the fact that the excess SOD activity happens to be of a mutant human variety.

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*Response*: We concluded from our data that the mutations of SOD found in familial ALS (1, 2) cause a gain-of-function (3). The mutated enzyme, when produced at high amounts in the brain and spinal cord of transgenic mice, causes motor neuron disease in a pattern that resembles human familial ALS, while the wild-type enzyme does not. We now have seen disease in two out of four lines of mice expressing the $Gly^{93} \rightarrow Ala$ mutation (our lines G1 and G20). In addition, Gordon and co-workers find that a second mutation of SOD found in affected families ($Gly^{85} \rightarrow Arg$), also causes disease when expressed in mice (4). We and others have studied wild-type human SOD overexpressed at comparable levels in transgenic mice, yet have not found clinical signs of motor neuron disease (3, 5). Some of these mice have been followed from zero to 2 years of age (5), whereas disease in most lines of mice expressing mutant SOD develops by 4 to 6 months of age (3, 4). Because multiple lines of mice expressing at least two different mutations of SOD develop motor neuron disease, while multiple lines of mice expressing wild-type human SOD do not, the hypothesis that variability in transgene expression underlies disease seems unlikely.

Our data do not address the important issue, raised by McCord and others, of the role that oxidative stress may play in familial ALS. The mutations found in affected families decrease SOD activity (2), whereas our mutant and wild-type transgenic lines have a three- to fourfold elevation in SOD activity (3). McCord cites literature showing that either an increase or decrease in SOD activity may cause oxidative damage. Such damage may be a cofactor in disease, but oxidative stress per se cannot account for our findings, as only the mutant enzyme causes clinical disease in transgenic mice.

At issue is what is meant by "gain-of-function." Have mutant forms of human SOD gained a de novo enzymatic function, or do mutations potentiate catalysis of a normally unfavorable side reaction to which motor neurons are selectively vulnerable? If the latter, then high expression of the wild-type enzyme may cause subclinical pathology. This was suggested earlier by the studies of Avraham and colleagues that document changes at the neuromuscular junction in mice with high expression of wild-type SOD (5). High expression of mutant SOD in mice causes vacuolar changes, mitochondrial cytopathology, and accumulation of filamentous aggregates in ventral horn neurons of the spinal cord (6).

Whether or not subclinical changes of a similar type might be occurring in mice that express high amounts of wild-type human SOD needs to be addressed. In addition to the dismutation reaction (i) $2H^+ + O_2^- \rightarrow H_2O_2$, SOD also catalyzes several alternate reactions including: (ii) the formation of hydroxyl radical from hydrogen peroxide (7) and (iii), the nitration of proteins on tyrosine residues by peroxynitrite (8). Such side reactions might be facilitated by mutation, and to a lesser extent, by high expression of the wild-type enzyme. The rate limiting step in reactions (ii) and (iii) may be the access of reactants to the copper catalytic center at the bottom of the active site channel (9). By relaxing constraints on the size of the active site channel, the mutations found in affected families might cause a "gain-of-function" by facilitating one or more of these alternative reactions (2, 10). Increases in superoxide radical or hydrogen peroxide that result from loss or elevation of SOD activity may act as cofactors in disease if they potentiate one of these reactions.

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