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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REFERENCES

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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 Gly95 → Ala mutation (our lines G1 and G20). In addition, Gordon and co-workers find that a second mutation of SOD found in affected families (Gly95 → 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 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 vacular 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) $2H^+ + O_2^- → H_2O_2$, 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). 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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