tinejad, *Methods Enzymol.* **198**, 440 (1991)]. Pellets were prepared from a solution of PLF (10 μg/ml), PRP (2.5 μg/ml), bFGF (0.15 μM), or placental conditioned medium (100 μg/ml). A single pellet was implanted in each rat cornea. Vessels were visualized by perfusion with colloidal carbon.

18. Placental tissue was isolated from Swiss-Webster mice (Harlan Breeding Laboratories, Indianapolis, IN) from day 8 through day 18 of gestation. Tissue minces were placed in culture in DMEM without serum, and the medium from each culture was collected 24 hours later. A culture contained tissue from several placentas obtained from one pregnant mouse, and two or three independent cultures were prepared for each time point. The samples of conditioned medium were centrifuged briefly to remove tissue fragments and then concentrated by centrifugation through a Centricon filter with a 10,000 molecular weight cutoff (Amicon, Beverly, MA). The samples were stored at −20°C until they were tested in the endothelial cell migration assay.
19. D. Jackson, O. Volpert, N. Bouck, D. I. H. Linzer, unpublished data.
20. Antibodies were affinity-purified from a polyclonal antiserum to PRP (*6*). Medium from cultured placental tissue obtained on day 12 of gestation was fractionated by nondenaturing polyacrylamide gel electrophoresis. After transfer to nitrocellulose, the region of the filter containing PRP was excised and incubated with the polyclonal antiserum. Bound antibodies were eluted in 0.2 M glycine-HCl (pH 2.8), neutralized, dialyzed against phosphate-buffered saline (PBS), and concentrated in a Centricon microconcentrator.
21. Mouse placentas from days 14 to 16 of gestation were isolated, rinsed in phosphate-buffered saline (PBS), and frozen in a dry ice–ethanol bath. The tissue was embedded in O.C.T. compound (Miles, Elkhart, IN), and 5-μm sagittal sections were mounted onto gelatin-coated slides. Sections were dessicated and stored at −80°C, transferred to 4°C 24 hours before use, and then dried at room temperature for 10 min. A Pap-pen (Research Products International, Mount Prospect, IL) was used to create fluid barriers around each section before the sections were washed once in 50 mM tris-HCl (pH 7.6), 2 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 140 mM NaCl, and 0.1% BSA (solution 1) for 15 min at room temperature, twice in PBS for 10 min at room temperature, and then incubated in solution 1 supplemented with 5 mM $MgCl_2$, 5 mM $CaCl_2$, and 5 mM $MnCl_2$ (solution 2) for 2 hours at 4°C. After this incubation, 20 μl of ^{125}I-labeled PLF [6000 cpm of protein iodinated with Iodogen (Pierce, Rockford, IL) and separated from iodine by Sephadex G-100 column chromatography] in solution 2, with or without unlabeled PLF (1 μg/ml), was placed on each section and incubated for 24 to 48 hours at 4°C. Samples were washed three times in ice-cold PBS and then exposed to 2 mM bis (sulfosuccinimidyl) suberate (BS^3; Pierce) for 15 min at 4°C to cross-link proteins; the cross-linking reaction was quenched with 100 mM tris-HCl (pH 7.6). Sections were washed three times in PBS, fixed in 5% paraformaldehyde (pH 7.6) for 5 min at room temperature, and then washed again three times in PBS. Pap-pen barriers were removed by treatment with xylenes for 5 min, and the sections were dehydrated through an ethanol step series and dried in a vacuum with desiccant for 1 hour. The slides were then coated with Kodak NTB-2 photographic emulsion, allowed to dry, and stored at 4°C for 2 weeks until they were developed.
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24. Placental sections were stained to identify endothelial cells before coating with emulsion. To inactivate endogenous horseradish peroxidase (HRP) activity, we incubated sections in a solution of 70% methanol and 3% H_2O_2 in PBS for 30 min at 4°C and then rinsed them three times in PBS. The sections were then blocked in 5% BSA in PBS for 30 min at 4°C, incubated with the biotinylated lectin B4 from *Bandeiraea simplicifolia* [BSI-B4 from Sigma, St. Louis, MO; J. Coffin *et al.*, *Dev. Biol.* **148**, 51 (1991)] for 30 min at 4°C, washed three times in PBS, and finally incubated with a 1:500 dilution of streptavidin conjugated to HRP for 30 min at 4°C. The samples were again washed three times in PBS, and the HRP activity was visualized by addition of 1 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 in PBS. After the sections were stained, they were processed as before for emulsion autoradiography.
25. We thank S.-J. Lee and D. Nathans for purified PLF protein and antibodies, J. Folkman for the bovine capillary endothelial cells, and R. Lamb, K. Mayo, and B. Wu for critical reading of the manuscript. Supported by grants from NIH to D.I.H.L. (HD29962 and HD24518) and to N.B. (CA52750) and by the Northwestern University P30 Center in Reproductive Biology (HD28048).

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TECHNICAL COMMENTS

Strontium Isotopes in Mid-Cretaceous Seawater

B. Lynn Ingram *et al.* (*1*) model a general decrease in mid-Cretaceous strontium (Sr) isotope ratios that occurred as a result of an increase in oceanic crustal production. Their model uses the emplacements of only three oceanic plateaus: the Ontong-Java, Manihiki, and Kerguelen. Ingram *et al.* conclude "that the crustal production rates proposed by Larson [*2*, *3*] . . . are far too large . . ." (p. 549) because I have previously proposed (*2*–*5*) that these events were part of a much larger increase in oceanic crustal production from other oceanic plateaus, and in particular, from increased spreading rates at oceanic ridges. The comparison by Ingram *et al.* of their model for these three oceanic plateaus to my more general compilations of oceanic crustal production (*2*–*5*) is incorrect because it is a comparison between different volumes, and also types, of oceanic crust. For this comparison they first calculated a conversion factor of 6×10^8 mol of hydrothermal Sr flux per 1 km^3 oceanic crustal production, which fits a Cretaceous "background" oceanic crustal production rate of 20 km^3 per year (Fig. 1). This rate includes all extrusive basalts, diabase dikes, and underlying intrusive gabbros in the entire crustal sections above the mantle for both oceanic plateaus and normal ridges. However, in their figure 3, Ingram *et al.* propose a model to match observed Sr isotope ratios based only on the extrusive basalt volumes (*6*) capping the Ontong-Java, Manihiki, and Kerguelen plateaus. Finally, they contrast these matches with a much larger Sr isotope anomaly calculated from my compiled volumes (Fig. 1) of both the extrusive and intrusive sections of both plateaus and ridges. In general, the ratio of extrusive to intrusive crust in both situations is about 1 to 4 or 1 to 5. Ingram *et al.* (*1*) use 10×10^6 km^3 and 8×10^6 km^3 for the extrusive basalt volumes of (i) Ontong-Java and Manihiki and (ii) Kerguelen plateaus, respectively. My estimates (included in Fig. 1) of the combined extrusive and intrusive volumes of these same features were 64×10^6 km^3 and 45×10^6 km^3 (*3*–*5*). Thus, one would expect that the model Sr isotope anomalies calculated using these two different sets of figures would be different, but the comparison is meaningless because Ingram *et al.* have compared apples to oranges, or more precisely, apples to apples *and* oranges.

It is unlikely that serious, systematic errors will reduce the mid-Cretaceous pulse in oceanic crustal production as shown in Fig. 1 because I (*2*) deliberately chose the radiometric time scale (*7*) with the longest mid-Cretaceous time interval to test rigorously for such a pulse. The choice of any other recently published time scale would increase the amplitude of this pulse, and the most recent time scale (*8*) would increase the amplitude by about 20% relative to the steady state baselevel. The total increase in mid-Cretaceous crustal production (Fig. 1) is about what is necessary to account for the 250 m of eustatic sealevel elevation (*9*) during that period.

The misuse of crustal volume data in the study by Ingram *et al.* (*1*) points out an interesting question regarding the source locations in the oceanic crust where Sr is leached by hot, hydrothermal fluids. Experimental results (*10*) suggest that fluid leaching diabase dike rock at 400°C matches the output of ridge crest hot springs and that basalts are much less susceptible to leaching. Field studies on ophiolites (*11*) confirm this result. Such source rocks and conditions probably are present directly above the 1.5 to 3 km deep (*12*) magma chambers at fast and intermediate spreading ridge crests. Fluid circulation must penetrate this deeply because convective cooling has depressed these magma chambers well below the 0.5-km level of neutral buoyancy (*12*–*14*). The situations for oceanic plateaus and

slow spreading ridges are less clear, but these may be less important sources because they would have more moderate geothermal gradients above their deeper magma chambers and because mid-plate plateaus lack a tensional tectonic environment to promote the cracking necessary for fluid penetration. Fluids may penetrate into the intrusive sections on ridge flanks, but the volumes and temperatures probably are too low to mobilize significant Sr. Excessive in situ pressure and the lack of tensional stress make it unlikely that the greatly thickened intrusive sections of oceanic plateaus could be permeated by any fluids (*15*). Thus, it is unlikely that the intrusive sections of either ridges or plateaus are significant sources of hydrothermal Sr, and the uppermost sections of their extrusive layers probably are also excluded by their temperature and rock type. The source location probably is confined to a layer about 1 km or less in thickness (*11*) near the extrusive-intrusive interface, and ridges with rates of spreading that are moderate to fast are probably the most important sources.

Thus, an alternate explanation of the mid-Cretaceous Sr isotope anomaly is that it is primarily a result of variations in crustal production from spreading ridges, and plateau production is unimportant. The percentage increase in hydrothermal flux [figure 3A in (*1*)] that was necessary to match their largest Sr isotope anomaly appears to be about 25% over the base value. The mid-Cretaceous anomaly in ridge production (Fig. 1) is about a 5 km^3 per year rate increase over a ridge baselevel of 18.5 km^3 per year (*4*). This increase to an average of 23.5 km^3 per year is 28% above the ridge baselevel, and thus is a close match to the largest increase calculated by Ingram *et al.* in hydrothermal flux. This approach follows that of Jones *et al.* (*16*) who worked with a more complete data set than the one in question (*1*), and produced a more quantified and successful model of Sr isotope ratio variations from 120 to 40 Ma (mid-Cretaceous to the Eocene) as a function of variations in area of oceanic crustal production from spreading ridges. Jones *et al.* also did not account for oceanic plateau production, and extracted their area parameter from my oceanic ridges histogram (Fig. 1) by dividing out the constant, 6.5 km thickness for that type of oceanic crust. Thus, my assumptions about the main sources of the hydrothermal flux are essentially equivalent to those of Jones *et al.* (*16*) and are in sharp contrast to those of Ingram *et al.* (*1*).

Such simple models may be complicated by a nonlinear dependency on spreading rate and at least two other problems. First, increased oceanic crustal production will increase continental weathering, and thus the oceanic Sr isotope ratio, by an unquantifiable amount because of processes proposed by Weissert (*17*). Thus, the observed Sr isotope ratio always should be higher than predictions based only on increases in the hydrothermal flux, as it would be in my above example. Second, the exact shape of the spreading-ridge anomaly in mid-Cretaceous oceanic crustal production is unknown because it occurred during the Cretaceous magnetic superchron when spreading rates must be averaged over 40 Ma. The general concept that oceanic crustal production controls hydrothermal Sr flux and thus influences oceanic Sr isotope ratios is almost certainly correct, but quantification of this controlling function is a complex and uncertain business.

Roger L. Larson
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882, USA

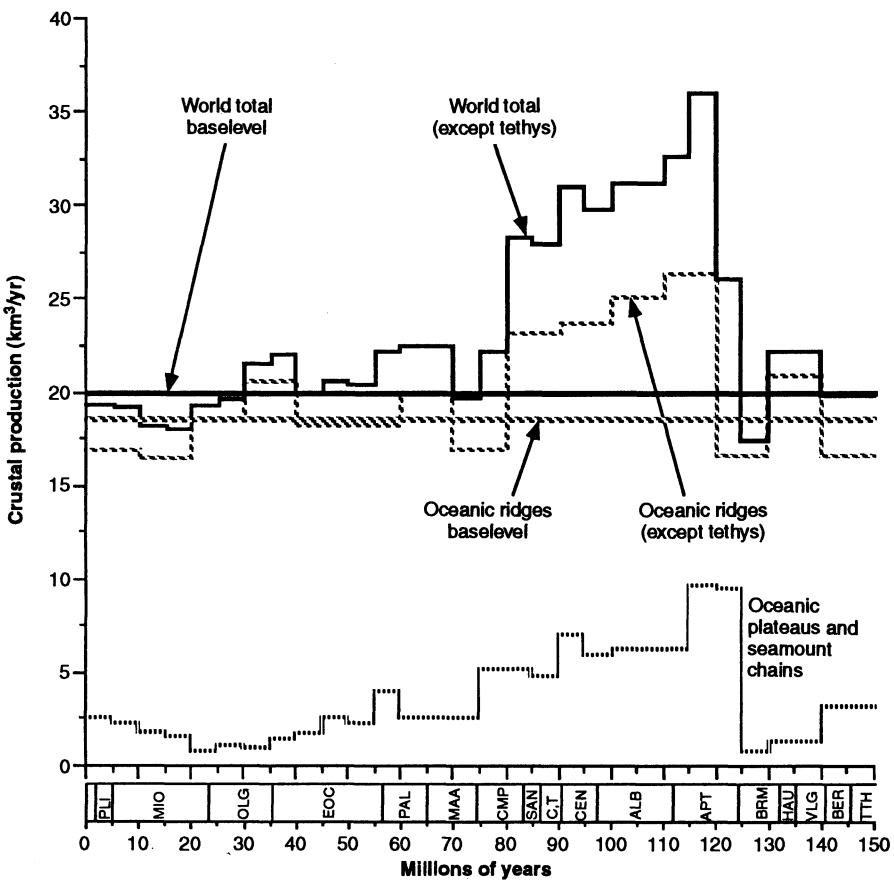

Fig. 1. World oceanic crustal production for the past 150 Ma, including all igneous extrusive and intrusive material above the Mohorovicic Discontinuity. The histograms for ridges (*2*) indicate crust formed by seafloor spreading, and the histogram for oceanic plateaus and seamount chains (*3–5*) indicate crust formed by mantle plumes. The world total histogram (*3*) is the sum of the other three histograms.

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18. I thank my "strontium support group," consisting of D. J. Allard, R. T. Bird, S. N. Carey, C. E. Jones, J. H. Natland, B. N. Opdyke, J. G. Schilling, and P. A. Wilson for their advice in preparing this technical comment.

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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 1.2×10^{10} mol per year (for 20 km^3 of total crust or 4 km^3 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.

B. Lynn Ingram
Department of Geology and Geophysics, University of California, Berkeley, CA 94720, USA
Frank M. Richter
Department of Geophysical Sciences, University of Chicago, Chicago, IL 60637, USA

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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-