

and Φ_{yy} can be estimated from the set of r and y example pairs.

23. Estimation of the operator on two-dimensional examples is possible, but computationally very expensive if done in the same way. The present computer simulations require several hours when run on standard serial computers. The two-dimensional case will need much more time (our one-dimensional estimation scheme runs orders of magnitude faster on a CM-1 Connection Machine System with 16K-processors).

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Seawater Strontium Isotopes, Acid Rain, and the Cretaceous-Tertiary Boundary

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A large bolide impact at the end of the Cretaceous would have produced significant amounts of nitrogen oxides by shock heating of the atmosphere. The resulting acid precipitation would have increased continental weathering greatly and could be an explanation for the observed high ratio of strontium-87 to strontium-86 in seawater at about this time, due to the dissolution of large amounts of strontium from the continental crust. Spikes to high values in the seawater strontium isotope record at other times may reflect similar episodes.

IN RECENT YEARS MUCH PROGRESS HAS been made in two areas of research that appear unrelated: precise documentation of the strontium isotopic composition of ocean water through time and the understanding of the atmospheric geochemical effects that accompany impacts of large extraterrestrial objects on the earth. Both may be important for understanding the events that occurred at the Cretaceous-Tertiary (K-T) boundary.

Hess *et al.* (1) recently presented new data on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in seawater over the past 100 million years. They analyzed selected fossil foraminifera from Deep Sea Drilling Project cores and showed that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio generally increases smoothly from near 0.7074 at 100 million years ago to the present-day seawater value of 0.7092. However, anomalously high ratios occur at the K-T boundary, forming a pronounced maximum in the otherwise relatively smooth curve. They considered whether a large impact, suggested as the cause of iridium anomalies at this boundary (2), could also be responsible for the strontium isotopic anomaly. Two possibilities were examined: (i) that the elevated $^{87}\text{Sr}/^{86}\text{Sr}$ ratio was due to dissolution of the bolide in seawater and (ii) that the impact ejecta (vapor and solid) were the major source of the strontium. However, Hess *et al.* (1) showed that although both continental crust and chondritic meteorites have high strontium isotopic

ratios, these sources could not supply sufficient strontium to account for the observed seawater increase.

Prinn and Fegley (3) analyzed the atmospheric chemical effects of the impact of a large projectile on the earth and, following an earlier idea by Lewis *et al.* (4), calculated that the major effect would be the production of large amounts of nitrogen oxides (NO_x) due to shock heating of the atmosphere. As a consequence, extremely acidic precipitation would occur immediately near the impact site, and, over a more extended time period, it would occur globally. The resulting enhanced weathering of the continents would increase the supply of continental strontium to the oceans, and potentially

might be a cause for the high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio observed at the K-T boundary. Even under a normal weathering regime the most important factor controlling strontium isotopic variation in seawater is the isotopic composition of the riverine strontium input (5).

Seawater strontium isotope data from three recent papers (1, 5, 6) for the time period from 50 million to 80 million years ago are plotted in Fig. 1. Age assignments for most of these samples are probably accurate to 1 million to 2 million years, although there may be more uncertainty attached to some of the non-Deep Sea Drilling Project samples analyzed by DePaolo and Ingram (6). Agreement among the three laboratories for samples of approximately the same age is generally good, and for modern shells and seawater the bias-adjusted values agree within reported uncertainties. Thus apparently "anomalous" values (Fig. 1) are probably not due to interlaboratory effects. The high values near the K-T boundary are apparent, although there is some scatter. One sample in particular [sample P3 measured by DePaolo and Ingram (6) and assigned an age of 62.5 million years] that is somewhat younger than the boundary nevertheless has a high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Comparison with the other isotopic data suggests that it may be a few million years older. The sample is a "Middle Danian" rhynchonellid brachiopod from Faxe in Denmark; this species was one of the first to appear in the Tertiary in this area (7). If this sample is reworked or if the age assignment is erroneously low, then the strontium isotopic spike appears to begin very abruptly at the K-T boundary, high values occur over a time period of about 2 million years, and the ratios then begin to decrease to pre-Tertiary values (Fig. 1). This is consistent with rapid introduction into the oceans of strontium

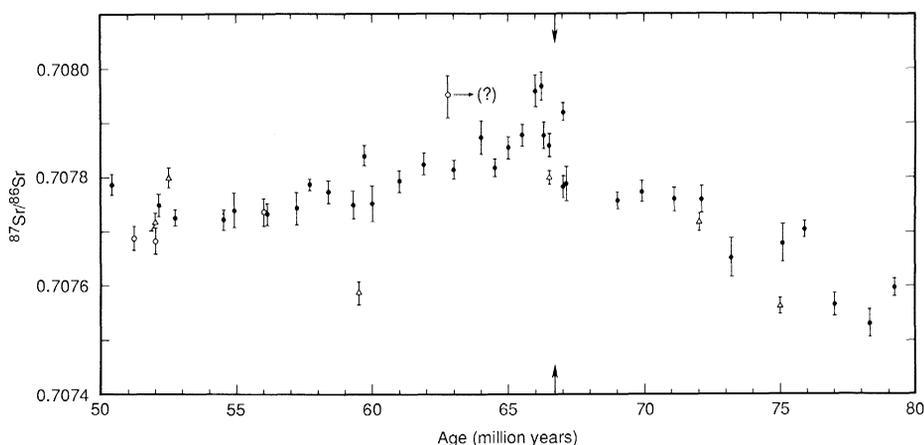


Fig. 1. Strontium isotopic composition of seawater for the period from 50 million to 80 million years ago, based on data from (1) (solid circles), (5) (open triangles), and (6) (open circles). Arrows on the time axis indicate the K-T boundary. The apparently anomalous sample P3 (question mark) is discussed in the text. All data are bias-adjusted to the $^{87}\text{Sr}/^{86}\text{Sr}$ value for National Bureau of Standards standard strontium given in (1); errors are as reported in the original works.

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with a high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and gradual return to steady state according to the residence time of strontium in the oceans, which is presently approximately 4 million years (8). Not plotted in Fig. 1 are the data of Burke *et al.* (9), who measured a large number of samples but presented data only in graphical form. Their data also show a clear spike at the K-T boundary, with strontium isotopic ratios ranging up to approximately 0.708.

If the abrupt increase in $^{87}\text{Sr}/^{86}\text{Sr}$ in seawater near the K-T boundary is the result of greatly increased continental weathering, the total amount of strontium required to be delivered to the oceans depends strongly on its isotopic composition. The necessary input amounts are given for a simple mixing model in Table 1. Two strontium isotopic values are considered for seawater immediately after the K-T boundary: (i) the average of the three highest ratios for the period from 63 million to 67 million years (0.70796) and (ii) the average of all values over this time period (0.70785). Three different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are tested for the riverine input: 0.7111 [near the present-day globally averaged value (10)], 0.712 [approximately the value estimated by Palmer and Elderfield (5) for silicate weathering 65 million years ago], and 0.7175 [estimated in (5) for present-day silicate weathering]. For comparison, input amounts are also shown for strontium with $^{87}\text{Sr}/^{86}\text{Sr} = 0.743$, an average of carbonaceous meteorite values from (11) and probably appropriate for a comet. A bolide of the size postulated in (3) would contain less than 0.1% of the strontium required for this case.

Could the strontium amounts in Table 1 be supplied to the oceans by acid rain-enhanced continental weathering? The values are very large, 1 to 6% of the present oceanic strontium inventory, and amount to 4×10^4 times the present annual input of strontium from rivers even for the lowest estimate. They are calculated on the basis of a Cretaceous oceanic strontium inventory equivalent to today's. Graham *et al.* (12) showed that the ratio of strontium to calcium in planktonic foraminifera was 15 to 20% lower during the Cretaceous than at the present, presumably reflecting a similar difference in seawater. They suggested that the difference is likely due to high hydrothermal extraction rates for calcium at earlier times; if instead calcium contents have remained approximately constant and the strontium content of Cretaceous seawater was 20% lower, then the amounts of strontium required would be lower by an equivalent amount.

Given the complex weathering regime, it is difficult to estimate accurately the amount

Table 1. Amounts of added strontium required to produce an increase ($\Delta 87$) in the seawater $^{87}\text{Sr}/^{86}\text{Sr}$ ratio at the K-T boundary as a function of the isotopic ratio of the input. $\Delta 87$ is relative to $^{87}\text{Sr}/^{86}\text{Sr} = 0.70776$, the average for the period from 68 million to 72 million years ago.

$\Delta 87$	Required strontium amount (moles) for various input $^{87}\text{Sr}/^{86}\text{Sr}$ ratios			
	0.711	0.712	0.7175	0.743*
1.98×10^{-4}	8.32×10^{15}	6.26×10^{15}	2.65×10^{15}	7.22×10^{14}
0.9×10^{-4}	3.65×10^{15}	2.77×10^{15}	1.19×10^{15}	3.27×10^{14}

*Assumed appropriate for a comet; see text for details.

of strontium that would be dissolved by even a known quantity of acid precipitation. Highly acidic rain would increase the rate of carbonate dissolution, but the radically different weathering effects of precipitation with pH 1 (3) would likely also increase the strontium contribution from silicate sources. Higher isotopic ratios would result as rubidium-rich phases such as micas became more susceptible to dissolution. There are no data available to assess this change quantitatively, but laboratory studies (13) show that decreasing pH over the range from 5 to 2 in weathering solutions can increase the amount of calcium (for example) removed from a granite by up to two orders of magnitude, and they also show that biotite mica is essentially completely removed during leaching with acidified ($\text{H}_2\text{SO}_4\text{-HNO}_3$) water at pH 2. Although relatively low in strontium content, micas such as biotite have generally high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and thus their enhanced dissolution has the potential to increase significantly riverine $^{87}\text{Sr}/^{86}\text{Sr}$ ratios.

Prinn and Fegley (3) considered two specific impacting bodies and calculated atmospheric NO production of 1.2×10^{17} moles (comet case) and 5×10^{14} moles (asteroid case). The largest uncertainties in these estimates are probably the mass and velocity of the bolide; there is also some uncertainty associated with the NO yield. At the extreme, the production rates could be underestimated by as much as two orders of magnitude, but almost certainly not more. Prinn and Fegley (3) estimated that between 0.015 and 40% of the NO amount would be rained out immediately in the vicinity of the impact. Taking the higher of the two production estimates and an intermediate value of 10% for immediate rainout, about 1.1×10^{17} moles of NO_x would remain in the atmosphere. About 23% of the present annual precipitation falls on the continents (14); if we assume a similar figure for the period after the impact, $\sim 2.5 \times 10^{16}$ moles of nitric acid would be available for continental weathering. This is only about 10 times the number of moles of strontium required to be added to the oceans (Table 1). On the other hand, at the extremes of the uncertainties in bolide kinetic energy, NO

yield, and immediate rainout discussed above, nitric acid available for continental weathering could exceed 10^3 times the number of moles of strontium required. This could dissolve enough strontium from limestone to satisfy the amounts required according to Table 1, but $^{87}\text{Sr}/^{86}\text{Sr}$ ratios would be too low (15). However, precipitation with very low pH is likely to have reacted selectively with minerals such as mica containing high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, greatly increasing input values. Any carbonate dissolution that did occur would have released CO_2 to the atmosphere, as would have acid added to the oceans (3). Although geologically transient, the additional atmospheric CO_2 would have had the effect of prolonging the period of enhanced continental weathering.

Thus, within the uncertainties of NO production, immediate rainout, and the weathering regime, it appears that acid precipitation after a large impact could account for the increase in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio at the K-T boundary. Such a rapid increase must almost certainly have been caused by the introduction of large amounts of strontium from the continental crust into the ocean. Indeed, the very large increase in the seawater $^{87}\text{Sr}/^{86}\text{Sr}$ ratio since the beginning of the Tertiary, about an order of magnitude greater than the K-T spike, has been attributed to increased silicate weathering (5), albeit over a much greater time span.

Increased volcanic activity near the time of the K-T boundary could also have led to acidic precipitation due to increased H_2SO_4 and HCl emissions (16). Prinn and Fegley (3) have argued that the environmental effects of such volcanism would be much less severe than those of impact-produced NO, largely because of the slow rate of volcanic acid addition. However, if comparable amounts of acid were injected into the atmosphere even over tens of thousands of years, the effects on seawater strontium probably could not be distinguished from the impact case because of the long oceanic residence time of strontium. This would be true only if continental precipitation remained acidic enough to enhance greatly both chemical weathering and the fraction of silicate-derived strontium in runoff water.

If the K-T boundary isotopic spike is indeed the result of impact-related acid rain, the oceanic strontium isotope record may reveal other large impacts. The seawater strontium curve of Burke *et al.* (9), which spans the past 500 million years, shows at least two other prominent high spikes in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio, one in the mid-Cretaceous, at ~100 million years, and the other in the Pennsylvanian, at ~290 million years. The first appears to precede by a few million years the mass extinction event at the Cenomanian-Turonian boundary. There is also a large increase in $^{87}\text{Sr}/^{86}\text{Sr}$ across the Permian-Triassic boundary (9), the time of the most extreme mass extinction in the Phanerozoic record (17). However, the increase appears to be rather gradual, extending over 20 million to 25 million years, and is thus quite different in character from the K-T spike. Nevertheless, data are sparse for this interval, and more work will be required to determine the exact nature of the increase.

The occurrence of a spike toward higher values in the seawater $^{87}\text{Sr}/^{86}\text{Sr}$ record at the K-T boundary is tantalizing evidence for enhanced continental weathering, possibly due to impact-related acid rain. Detailed strontium isotopic studies through this and other intervals where such spikes appear are required to determine precisely the nature of the isotopic variations with respect to stratigraphy, and particularly with respect to mass extinctions.

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Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase

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A thermostable DNA polymerase was used in an in vitro DNA amplification procedure, the polymerase chain reaction. The enzyme, isolated from *Thermus aquaticus*, greatly simplifies the procedure and, by enabling the amplification reaction to be performed at higher temperatures, significantly improves the specificity, yield, sensitivity, and length of products that can be amplified. Single-copy genomic sequences were amplified by a factor of more than 10 million with very high specificity, and DNA segments up to 2000 base pairs were readily amplified. In addition, the method was used to amplify and detect a target DNA molecule present only once in a sample of 10^5 cells.

THE ANALYSIS OF SPECIFIC NUCLEOTIDE sequences, like many analytic procedures, is often hampered by the presence of extraneous material or by the extremely small amounts available for examination. We have recently described a method, the polymerase chain reaction (PCR), that overcomes these limitations (1, 2). This technique is capable of producing a selective enrichment of a specific DNA sequence by a factor of 10^6 , greatly facilitating a variety of subsequent analytical manipulations. PCR has been used in the examination of nucleotide sequence variations (3-5) and chromosomal rearrangements (6), for high-efficiency cloning of genomic sequences (7), for direct sequencing of mitochondrial (8) and genomic DNAs (9, 10), and for the detection of viral pathogens (11).

PCR amplification involves two oligonucleotide primers that flank the DNA segment to be amplified and repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences, and extension of the annealed primers with DNA polymerase. These primers hybridize to opposite strands of the target sequence and are oriented so DNA synthesis by the polymerase proceeds across the region between the primers, effectively doubling the amount of that DNA segment. Moreover, since the extension products are also complementary to and capable of binding primers, each successive cycle essentially doubles the amount of DNA synthesized in the previous cycle. This results in the exponential accumulation of the specific target

fragment, approximately 2^n , where n is the number of cycles.

One of the drawbacks of the method, however, is the thermolability of the Klenow fragment of *Escherichia coli* DNA polymerase I used to catalyze the extension of the annealed primers. Because of the heat denaturation step required to separate the newly synthesized strands of DNA, fresh enzyme must be added during each cycle—a tedious and error-prone process if several samples are amplified simultaneously. We now describe the replacement of the *E. coli* DNA polymerase with a thermostable DNA polymerase purified from the thermophilic bacterium, *Thermus aquaticus* (Taq), that can survive extended incubation at 95°C (12). Since this heat-resistant polymerase is relatively unaffected by the denaturation step, it does not need to be replenished at each cycle. This modification not only simplifies the procedure, making it amenable to automation, it also substantially improves the overall performance of the reaction by increasing the specificity, yield, sensitivity, and length of targets that can be amplified.

Samples of human genomic DNA were subjected to 20 to 35 cycles of PCR amplifi-

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