Reports

Racemization of Amino Acids in Marine Sediments

Abstract. *Isoleucine, one of several amino acids isolated from a suite of well-dated deep-sea cores, shows a progressive increase in the degree of racemization with the age of the sediment. Amino acids in sediments show an initial rate of racemization almost an order of magnitude faster than the rate observed for free amino acids at a comparable* pH *and temperature. The observed kinetics depend on a variety of diagenetic processes, but it appears that the ratio of alloisoleucine to isoleucine is a reliable indicator of age for samples less than 400,000 years old; for older samples the results are more ambiguous. Isoleucine is racemic in samples older than about* 15×10^6 *years.*

Proteins, peptides, and amino acids can undergo a variety of biological and nonbiological reactions in the geologic environment. Biological amino acids, the basic components of proteinaceous matter, are primarily of the L form, and with time these optically active enantiomers convert to an equilibrium mixture of the D and L forms. This phenomenon has now been observed in several apparently diverse environments: in fossil calcareous organisms from former nearshore marine sediments (*1–3*); in nearshore fjord-type sediments (*4*); and deep-sea marine sediments (*2*, *5*). In fossil shells from several Recent, Pleistocene, and Tertiary deposits from the southeastern United States, racemization was found to increase with the increasing age of the fossil, with complete racemization found in fossils older than the Pliocene (*1*, *2*). Fossil corals from raised reef deposits on Barbados likewise showed increased racemization in fossils from older terraces, though in situ contamination apparently occurred in some samples (*3*). Kvenvolden *et al.* (*4*) found that racemization in the sediments of Saanich Inlet had proceeded to about 10 percent of the amount at equilibrium in the first 9000 years of diagenesis. Hare and Mitterer (*2*) found that racemization of isoleucine in an Antarctic deep-sea core increased with depth in the core and that, when compared to the sedimentation rates determined from paleomagnetic data, the rate of racemization of isoleucine was in good agreement with the rates extrapolated from high-temperature pyrolysis data on actual shell material. Recently Bada *et al.* (*5*) have found a progressive increase in the ratio of alloisoleucine to isoleucine with depth in a core from the Mid-Atlantic Ridge. High-temperature pyrolysis experiments on solutions of free L-isoleucine were used to calculate a sedimentation rate of 0.42 cm per 1000 years for this core.

In this work we report data on the racemization of L-isoleucine in a suite of cores of deep ocean sediments; the ages of all samples analyzed are known independently. We offer here a mechanism to explain the observed kinetics of racemization, and we shall reinterpret existing data (*5*) in light of this mechanism.

The racemization of L-isoleucine produces D-alloisoleucine (*6*); because of their two adjacent asymmetric carbon atoms, these diastereomers have slightly different chemical properties and are resolvable in the standard ion-exchange procedure of amino acid analysis. Thus the measurement of the extent of racemization of isoleucine is comparatively simple. It has been shown (*1*, *2*, *4*, *5*, *7*) that isoleucine is one of the slowest amino acids to racemize, so it provides an index of the degree of racemization of other amino acids in a sample (*8*).

We have analyzed the foraminifera fraction (> 74 μm) rather than the gross sediment, the rationale being that the mineral test would be most resistant to contamination from outside sources. Samples are ultrasonically cleaned, washed, wet-sieved with distilled water, air-dried, and weighed. They are then dissolved in twice the stoichiometric amount of concentrated HCl, and a slight excess of acid is added to make the final solution 6*N* HCl. Hydrolysis is performed in sealed Pyrex test tubes under N_2 for 22 hours at 110°C. Desalting techniques used

Table 1. Cores analyzed [see (*22*) for a discussion of dating by ^{230}Th]. Cores V23-110 and V24-28 have only faunal analyses for their age determinations. Core V23-110 is judged to be about 2.0×10^6 years old at the core bottom (821 cm) on the basis of the presence of *Globorotalia multicamerata*, *Sphaeroidinella dehiscens*, and left-coiling *Pulleniantina primalis* at the 800-cm level, and the abundance of discoasters below 780 cm (*26*). Core V24-28 is entirely Upper Pleistocene in age, and faunal analyses and correlations with other cores indicate an age of about 400,000 years at the core bottom, 910 cm (*27*). All cores listed have been obtained on cruises of Lamont-Doherty Geological Observatory research vessels, with the exception of core CH96-G12, which was obtained by the Woods Hole Oceanographic Institution.

Core	Latitude	Longitude	Water depth (m)	Approximate water temperature (°C)	Method of dating	Reference
V12-122	17°00′N	74°28′W	2730	4.0	^{14}C, ^{230}Th, fauna	(*28*, *29*)
A179-4	16°36′N	74°48′W	2965	4.0	^{14}C, ^{230}Th, fauna	(*28*)
V16-205	15°24′N	43°24′W	4045	2.4	Magnetic reversals, fauna	(*28*)
V16-39	24°43′S	4°45′W	4510	2.4	Magnetic reversals, fauna	(*28*)
V12-18	28°42′S	34°30′W	2935	2.9	Magnetic reversals, fauna	(*28*)
RC8-93	29°22′S	105°14′W	3157	2.0	Magnetic reversals	(*28*)
V23-110	17°38′N	45°52′W	3746	2.4	Fauna	(*26*)
CH96-G12	30°16′N	43°19′W	4014	2.2	^{230}Th, fauna	(*5*, *13*)
V24-28	15°19′N	77°57′W	2274	4.1	Fauna	(*27*)
RC5-12	26°35′N	56°29′W	5104	1.5	Fauna	(*26*)
A167-21	29°49′N	76°35′W	1455	~5	Fauna	(*30*)
V18 BBD No. 2	17°56′S	154°08′W	~ 800	~5	^{14}C	This work

Table 2. Alloisoleucine, isoleucine, and the alloisoleucine to isoleucine ratio in hydrolyzates of foraminifera from deep-sea cores.

Depth in core (cm)	Approximate age ($\times 10^6$ yr)	Alloisoleucine (nmole/g)	Isoleucine (nmole/g)	Alloisoleucine / Isoleucine
		A179-4		
30	0.013	2.69	64.84	0.042
100	.042	3.82	39.72	.096
100	.042	2.62	28.20	.093
175	.073	6.15	54.20	.114
250	.105	6.50	37.50	.172
403	.169	11.30	55.40	.204
403	.169	12.80	65.60	.195
600	.250	13.27	35.22	.377
700	.295	12.90	35.10	.368
		V23-110		
0–1	>0	2.1	35.7	.059
21–22	~ 0.05	3.7	41.6	.089
42–43	~ .10	6.0	39.5	.152
62–63	.16	7.7	37.5	.205
62–63*	.16	4.1	17.7	.232
81–82	.20	8.0	31.6	.253
101–102	.25	7.7	27.0	.285
151–152	.38	12.2	41.4	.295
211–212	.53	13.0	38.3	.339
301–302	.75	11.6	33.4	.347
405–406	1.01	8.2	21.4	.383
405–406*	1.01	7.2	19.6	.367
499–500	1.24	10.4	29.7	.350
591–592	1.48	5.2	10.6	.491
682–683	1.71	11.8	26.0	.459
791–792	1.90	9.5	15.0	.633
		V12-122		
40–41	0.017	5.2	72.8	.071
98–99	.041	8.0	66.7	.120
98–99*	.041	7.1	44.2	.161
290–291	.121	11.8	56.4	.209
290–291*	.121	15.6	62.4	.250
464–465	.193	8.5	34.6	.255
505–506	.210	10.0	37.2	.269
551–552	.230	10.0	44.6	.224
650–651	.271	12.0	38.9	.309
745–746	.310	8.9	28.7	.310
850–851	.355	7.8	25.5	.306
950–951	.395	11.5	28.4	.405
1055–1056	.438	10.1	28.5	.354
1097–1098	.456	11.0	33.7	.326
		V16-205		
375	.70	7.18	16.85	.43
375	.70	10.45	22.41	.46
861	1.80	10.24	16.83	.609
		V16-39		
290	0.700	5.56	15.38	.387
730	1.80	4.71	7.47	.63
		V12-18		
720	1.80	3.56	6.73	.53
		RC8-93		
1087	2.20	1.90	2.99	.637
		CH96-G12		
400	~ 0.10	2.85	18.08	.158
		V24-28		
5–6	~ .003	1.13	72.78	.016
413–416	~ .170	9.82	44.50	.220
		V18- BBD No. 2		
Dredge haul	.010	2.4	33.5	.072
Dredge haul	.010	1.3	17.9	.073
		RC5-12		
317–320	~ 70	4.60	3.44	1.33
317–320	~ 70	3.07	2.27	1.35
317–320	~ 70	1.14	1.42	0.80†
		A167-21		
300	~ 50	3.61	4.89	.74†
		JOIDES samples		
Leg 7, site 62-1, Upper-Upper Miocene		2.54	2.35	1.08
Leg 7, site 64-1, Lower-Middle Miocene		3.9	3.3	1.18
Leg 6, site 47-2, Lower Paleocene		1.55	1.23	1.26

* Hydrolyzed for 46 hours. † Not subjected to ultrasonic treatment.

are those of Hare (*9*). The sample size is usually on the order of 200 mg of foraminifera; thus reagent blanks are kept to a minimum. Negligible amounts of all amino acids except serine and glycine are found in blanks carried through the entire procedure. All cores analyzed are described in Table 1: data are given for the location, depth, bottom water temperature, and method used for the determination of the absolute age. The rate of racemization is sensitive to temperature (*1, 2, 5, 7*); all cores discussed have bottom-water temperatures in the range from 2.2° to 4.0°C. The present-day temperature is taken as an estimate of the average thermal condition of the cores; we have not corrected any of the data for temperature differences between cores because of other uncertainties. For large temperature differences (≈ 3°C), some correction would be necessary.

Data for the alloisoleucine to isoleucine ratio in these samples are given in Table 2; age assignments are given by interpolation or extrapolation from dated horizons. We report the absolute abundances of the amino acids with some caution, for consistent percentage recovery is not easily attained (*10*). Duplicate analyses indicate an uncertainty of up to $\pm$ 30 percent for the absolute abundance of any amino acid and an uncertainty of $\pm$ 5 percent in the alloisoleucine to isoleucine ratio. In some cases the ratios scatter even more than this in successive levels in a core, particularly in samples from core V12-122.

Racemization during acid hydrolysis is a potential problem (*4, 11*); extended hydrolysis (46 hours) significantly increases the alloisoleucine to isoleucine ratio in three out of four samples tested. However, little or no alloisoleucine is observed in samples of living corals analyzed by identical techniques (*3*); samples of living foraminifera have not been analyzed (*12*), but the presence of alloisoleucine in the sample from the top of core V23-110 is probably due primarily to the finite age of the top of the core. The youngest sample analyzed, from core V24-28, shows an alloisoleucine to isoleucine ration of ~ 0.015.

The kinetics of racemization in simple systems have been observed to obey first-order reversible rate laws (*5*); for the racemization of isoleucine, the equation is:

$$\text{L-isoleucine} \underset{\lambda_A}{\overset{\lambda_I}{\rightleftharpoons}} \text{D-alloisoleucine} \quad (1)$$

where λ_I and λ_A are the characteristic rate constants of isoleucine (I) and alloisoleucine (A), respectively. Alloisoleucine is more abundant than isoleucine in the equilibrium mixture of the two diastereoisomers. The equilibrium ratio has been found to be between 1.25 and 1.40 in high-temperature heating experiments (*1, 2, 7, 11*) and in old fossils at normal environmental temperatures (*1, 2*). There is no definite evidence of a temperature dependence of this ratio, although a slight one might be expected (*13*). We use a value of 1.25 for calculations here. Thus the ratio of the rate constants is fixed: $\lambda_I/\lambda_A = 1.25$. For the equation

$$\frac{dI}{dt} = -\lambda_I(I) + \lambda_A(A) \quad (2)$$

$$\frac{X_e - X}{X_e} = e^{-2.25\lambda_A t} \quad (3)$$

The quantity X_e is the equilibrium ratio of alloisoleucine to (alloisoleucine + isoleucine), or $1.25/(1.25 + 1.0) = 0.556$; X is the value of this ratio at time t (*14*). If the data fit this model a straight line for the plot of the logarithm of $(X_e - X)/X_e$ as a function of time would result; the data are plotted in Fig. 1, and clearly such a straight-line relation does not exist. The plot is linear to an alloisoleucine to isoleucine ratio of about 0.25 (approximately 400,000 years at these temperatures), and then the rate of racemization decreases rather abruptly. Similar conclusions can be drawn about racemization in fossil corals (*3*) and fossil *Mercenaria* (*1, 2*); the change in slope occurs at about 50,000 years at the higher temperatures. Contamination of older samples could give the curve this shape, but we feel that the major explanation for this nonlinearity comes from an understanding of the complexity of the diagenetic reactions that fossil amino acids undergo.

When the foraminifera are dissolved and the hydrolysis step is omitted, only free amino acids and small peptides are detected. In all cases, we observe that the alloisoleucine to isoleucine ratio is significantly higher in these free amino acids than in the total hydrolyzate (*15*). The free alloisoleucine to isoleucine ratio (Table 3) reaches a value of 1.0 in samples as young as 5000 years; this ratio increases up to a value of 1.35 to 1.40 in samples of Miocene age (*3*). Significant racemization of free amino acids was first observed in younger fossil *Mercenaria* samples (*1, 2*).

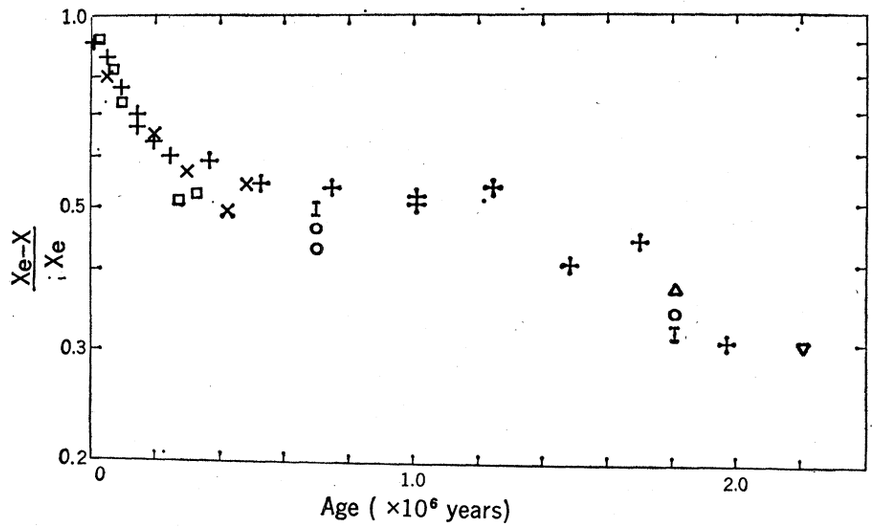

Fig. 1. Plot of $(X_e - X)/X_e$ as a function of time in deep-sea sediments. +, Core V23-110; □, core A179-4; ×, core V12-122; ○, core V16-205; △, core V12-18; ▽, core RC8-93; I, core V16-39.

Free amino acids represent a very small fraction of the total amino acids in young fossils (*1, 2*), but the ratio of free amino acids to total amino acids grows slowly with time, approaching a value of 1.0 (*16*). Almost all the free amino acids in a fossil are derived from the breakdown of proteins, and their extensive racemization is apparently a consequence of the natural hydrolysis of peptide bonds at the slightly alkaline *p*H's of the carbonate environment (*17, 18*). Hydrolysis under these conditions does not produce completely racemic amino acids, however, and, once liberated, these free amino acids continue to racemize at their own characteristic rates. Laboratory pyrolysis experiments indicate that racemization in calcareous matrices can be inhibited by a deficiency of water (*2*), further suggesting the link between the hydrolysis and racemization mechanisms. These experiments also indicate that amino acids are leached from the fossil, so that the rate of removal of amino acids from the matrix will also affect the abundances of alloisoleucine and isoleucine in the fossil (*19*). The free amino acids leached from both corals and foraminifera have alloisoleucine to isoleucine ratios of 0.95 to 1.3 whereas the hydrolyzates of the material leached have alloisoleucine to isoleucine ratios nearly identical to the ratios found in the hydrolyzates of the residual sam-

Table 3. Free amino acids in selected samples.

Depth in core (cm)	Alloisoleucine (nmole/g)	Isoleucine (nmole/g)	Alloisoleucine / Isoleucine
	V23-110		
0–1	0.4	0.3	1.3
42–43	1.7	1.5	1.13
62–63	4.1	4.1	1.0
81–82	6.2	5.7	1.1
151–152	2.7	2.6	1.04
405–406	6.9	6.9	1.0
591–592	1.4	1.5	0.93
791–792	1.7	1.8	.94
	V12-122		
40–41	0.3	1.1	.27
505–506	6.8	7.2	.97
650–651	7.7	7.9	.98
850–851	7.6	7.6	1.0
1055–1056	9.1	8.4	1.1
	V24-28		
5–6	~ 0.32	0.32	~ 1.0
	JOIDES samples		
Leg 7, site 64-1, Lower-Middle Miocene	1.6	1.1	1.45
Leg 6, site 47-2, Lower Paleocene	1.46	1.09	1.33

ples. The total amino acid content of the foraminifera studied here varies from about 1500 nmole/g in the youngest samples to about 500 nmole/g in Lower Pleistocene samples, most of the decrease coming in the first 200,000 years of diagenesis. Free amino acids accumulate in the fossils up to a concentration of about 350 nmole/g when the alloisoleucine to isoleucine ratio in the total hydrolyzate is in the range from 0.25 to 0.30. The abundance of free amino acids slowly decreases beyond an age of about 400,000 years. From the shape of the curve in Fig. 1, we conclude that rapid racemization during early diagenesis is a consequence of natural hydrolysis and the accumulation of nearly racemic free amino acids in the fossil. As indicated by the ratio of free amino acids to total amino acids, hydrolysis is not completed within 500,000 years at these temperatures; beyond this time, the rate of racemization is probably controlled by slow hydrolysis, continued racemization of free amino acids, and some racemization of bound amino acids. The exact mechanism of this latter process is somewhat obscure, but evidence that it occurs does exist (*18*). Presumably terminal amino acids would be free to racemize. Our data suggest that alloisoleucine is more abundant in the total (hydrolyzed) amino acids than in the free amino acid fraction, and earlier results (*1, 2, 4*) also suggest that racemization of amino acids does occur while they are bound. A simple calculation of the alloisoleucine to isoleucine ratio in the "peptide" fraction

$$\frac{(A)_{total} - (A)_{free}}{(I)_{total} - (I)_{free}} \qquad (4)$$

is vulnerable to the uncertainties of percentage recovery discussed above, but it appears that the kinetics of racemization in the "bound" fraction are nearly linear and not too different from the rates based on laboratory experiments (*5*). Ideally one should measure the extent of racemization only after all free amino acids have been removed (*4*).

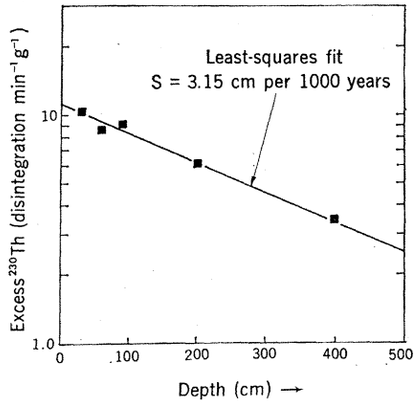

Fig. 2. Least-squares plot of excess ^{230}Th as a function of depth for core CH96-G12. *S*, sedimentation rate.

Extrapolation of our data would lead to the conclusion that the racemization of isoleucine would be complete within 15×10^6 to 20×10^6 years. Analyses of several high-carbonate samples from the JOIDES (Joint Oceanographic Institutions for Deep Earth Sampling) Deep Sea Drilling Program, all stored frozen since collection, support this extrapolation (see Table 2). The fact that the alloisoleucine to isoleucine ratio for the free amino acids in the Lower Middle Miocene sample is somewhat higher than that for the total hydrolyzate suggests that true equilibrium has not been reached. The Paleocene sample is in equilibrium.

As the overall abundance of amino acids decreases, the potential effect of contamination increases. Contamination can obviously have the effect of indicating an "apparent" rate of racemization that is slower than the real rate (*4, 20*). Analyses of Eocene and Cretaceous samples (Table 2) indicate that "nonequilibrium" ratios can be found if the sample is not correctly prepared. The Eocene sample consisted mostly of small (< 74 μm) particles that could not be ultrasonically cleaned because of the loss of starting material that is inherent to this procedure. The Cretaceous sample, however, was rich in coarse, well-preserved foram tests which could be subjected to the standard vigorous ultrasonic treatment. Two analyses of hydrolyzates of this sample showed alloisoleucine to isoleucine ratios of 1.33 and 1.35, whereas a third sample that was merely rinsed with H_2O and dilute HCl showed a ratio of 0.80. None of the Lamont cores examined here have been stored frozen, and contamination during storage could be responsible for some of the scatter in the data. Because our extrapolation is supported by the JOIDES data and because we are able to observe racemic mixtures in samples that have not been stored or prepared in an unusual manner we are confident that contamination during storage has not systematically affected the results. The fact that we do see the effects of contamination points out the difficulties of detecting racemic mixtures in samples that have been exposed to terrestrial processes (*3, 4, 20, 21*).

The nonlinear kinetics and the initial rate of racemization that we have observed are not in agreement with the recent work of Bada *et al.* (*5*), and we will offer additional evidence that their data support the mechanism that we have discussed. They observed alloisoleucine to isoleucine ratios increasing monotonically to a value of 0.157 in the 500-cm depth of the core they examined, CH96-G12. An age of about 1.2×10^6 years was assigned to the bottom of the core (518 cm) on the basis of an extrapolation of the results of laboratory measurements of the rates of racemization of free isoleucine in buffered solutions at elevated temperatures (*5*). The alloisoleucine to isoleucine ratios were measured on hydrolyzates of total sediment samples. Our data show that the time constant for the racemization of free isoleucine is not an alloisoleucine to isoleucine ratio of peptide-bound amino acids. We observe an alloisoleucine to isoleucine ratio of

Table 4. Data on uranium and thorium isotopic composition of selected samples from core CH96-G12. Calcium carbonate samples were analyzed gasometrically; U and Th analyses were performed on totally dissolved samples by the techniques of Ku (*22*); dpm, disintegrations per minute; ppm, parts per million.

Depth (cm)	$CaCO_3$ (% by weight)	U (ppm)*	$\frac{^{234}U}{^{238}U}$	^{232}Th (ppm)*	^{230}Th (dpm/g)	^{230}Th excess† (dpm/g)
30	73			3.93 ± 0.25	10.8 ± 0.4	10.4 ± 0.4‡
60	71	0.63 ± 0.06	1.05 ± 0.11	3.76 ± 0.19	9.16 ± 0.25	8.68 ± 0.30
90	62	0.71 ± 0.04	1.03 ± 0.07	5.16 ± 0.26	9.68 ± 0.36	9.15 ± 0.40
200	79	0.46 ± 0.05	0.93 ± 0.12	2.22 ± 0.12	6.49 ± 0.20	6.16 ± 0.24
400	76	0.60 ± 0.02	0.92 ± 0.03	3.62 ± 0.20	3.87 ± 0.15	3.47 ± 0.17

* On a total sediment basis. † ^{230}Th excess = $^{230}Th_{total}$ (dpm/g) − U^{234} (dpm/g). ‡ Calculated on the assumption that [U] ($CaCO_3$-free) = 2.18 ± .22 (average of four other samples) and $^{234}U/^{238}U = 1.00$; we were unable to measure [U] in this sample because of very low recovery.

0.15 to 0.16 in samples about 125,000 years old, which would mean that core CH96-G12 has a sedimentation rate almost an order of magnitude greater than suggested by Bada *et al.* (*5*). Since the magnetic record is rather ambiguous (*5*), no definitive independent age estimates are available for this core. We undertook to obtain such information in order to provide a basis for comparison with our work. By the method of excess ^{230}Th (*22*) we conclude that core CH96-G12 has a sedimentation rate of about 3.1 cm per 1000 years, rather than the 0.42 cm per 1000 years proposed by Bada *et al.* Data are given in Table 4 and plotted in Fig 2. Calculations on the basis of excess ^{230}Th ($CaCO_3$-free) or excess $^{230}Th/^{232}Th$ give slightly higher sedimentation rates but somewhat poorer graphical plots. The coccolith *Emiliania huxleyi* (formerly *Coccolithus huxleyi*) is abundant at the 200- and 400-cm levels of core CH96-G12 (*23*), thus suggesting a sedimentation rate of at least 1.5 cm per 1000 years, since *E. huxleyi* made its evolutionary appearance about 250,000 years before the present (*24*). A high sedimentation rate for this core is not unreasonable since it is a core with a high-carbonate content (Table 4) taken from the floor of the Atlantis fracture zone, where localized "ponding" effects could be operative (*25*). The magnetic record of core CH96-G12 shows no reversals of inclination (*5*), thus suggesting that the age of the bottom of the core could be less than 690,000 years. Since core CH96-G12 has a high carbonate content, results of gross sediment analyses probably are similar to those that we have reported here for foraminifera. We analyzed the foraminifera from the 400-cm level of core CH96-G12 and found an alloisoleucine to isoleucine ratio of 0.158, as compared to the value of 0.127 found for the hydrolyzate of the total sediment samples (*5*). Without further analyses, it is difficult to attribute too much significance to the difference between the two results, although in situ contamination could be responsible for the difference. The data that we have obtained for core CH96-G12 put the results of Bada *et al.* (*5*) in substantial agreement with our own. If core CH96-G12 had as slow a sedimentation rate as originally calculated (*5*), we would predict that the "nonlinear" phenomenon would have been observed.

The above discussion indicates that the racemization of protein-derived amino acids in the geologic environment is not a simple process. The kinetics of racemization observed in hydrolyzates of fossil or sediment samples depend on a variety of time constants, none of which is easily evaluated. The rate of racemization of amino acids in both free and bound fractions, the rate of hydrolysis of peptide bonds, and the rates of diffusion of native or contaminating amino acids into or out of the system are all disguised in the simple mathematical expression of Eq. 2. Faced with the large number of parameters that can affect the apparent rate of racemization, we have chosen an entirely empirical approach and analyzed samples of known absolute age. In the present state of the art, reasonably reliable age estimates are available back to 400,000 years at deep-sea temperatures, and future work should concentrate on resolving the ambiguity of older samples. In any event, the use of racemization as a chronological tool will depend on a thorough understanding of the diagenetic reactions of amino acids in the particular system under study.

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6. Strictly speaking, this process is not racemization but epimerization. We use the term "racemization of isoleucine" rather loosely, referring only to the rearrangement about the α-carbon atom of the amino acid. We use the extent of epimerization of isoleucine as a measure of the degree of racemization of all the amino acids.
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8. We are presently determining the degree of racemization of other amino acids in these cores, in cooperation with K. A. Kvenvolden and E. Peterson.
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10. Tracers of norleucine, an amino acid not found in proteins, have been added to most of the samples. Generally recovery is between 70 and 90 percent, but occasionally recoveries as low as 20 percent are observed. Because of these problems, we do not place too much emphasis on the absolute abundance of any amino acid; ratios of amino acids are not susceptible to this problem, for the difficulty is entirely one of mechanical losses in the desalting, rather than selective degradation of particular amino acids.
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12. Analyses of 15 other core tops by similar techniques also show little or no alloisoleucine (<1 percent) (K. King, personal communication).
13. By analogy with isotopic equilibrium, a simple $1/T$ relation might be expected:
$$(\text{Allo/Iso})_{eq} = 1 + \sim 109/T \text{ (in °K)}$$
The constant 109 is fitted to the observed data. Equilibrium ratios no lower than 1.25 have been observed at 165°C, and ratios no higher than 1.4 have been observed at ambient temperatures.
14. Mathematics have been adapted from E. S. Gould, *Mechanism and Structure in Organic Chemistry* (Holt, Rinehart & Winston, New York, 1959).
15. Peptides eluting in the alloisoleucine position would lead us to an incorrect conclusion regarding the free amino acids [see P. Hamilton, *Anal. Chem.* **35**, 2055 (1963)]; K. A. Kvenvolden (personal communication) has recently confirmed that other free amino acids besides isoleucine are extensively racemized in these samples. These data will be the subject of a forthcoming paper.
16. Exactly when this ratio reaches this value is not known, nor is the exact nature of the bound amino acids in ancient fossils very clear (*1, 2, 9, 31*). We refer to the bound amino acids as "peptides" only very casually, for we have not confirmed the presence of peptides in the older samples.
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