catalytic activity of the carboxyl proteases and may be inactive or only weakly active. The ancestor of the hepadnaviruses may have acquired a second protease, at the NH_2 terminus of the core gene, to compensate for the mutation at the active site of the original protease.
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Calcium-41 Concentration in Terrestrial Materials: Prospects for Dating of Pleistocene Samples

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Calcium-41 has been suggested as a new tool for radiometric dating in the range of 10^5 to 10^6 years. The concentration of cosmogenic calcium-41 in natural samples of terrestrial origin has now been determined by high-sensitivity accelerator mass spectrometry after pre-enrichment in calcium-41 with an isotope separator. Ratios of calcium-41 to total calcium between 2×10^{-14} and 3×10^{-15} were measured for samples of contemporary bovine bone and from limestone deposits. Some prospects for the use of calcium-41 for dating Middle and Late Pleistocene bone and for other geophysical applications are discussed.

THE MEASUREMENT OF NATURAL concentrations of ^{41}Ca (half-life $t_{1/2} \cong 1.0 \times 10^5$ years) (*1*) has been proposed as a new tool for radiometric dating (*2, 3*) and as a geological solar-neutrino detector (*4*). The major interest in ^{41}Ca stems from its potential for establishing the date of calcium-containing materials to earlier times than is possible with ^{14}C ($t_{1/2}$ = 5730 years). Particular interest arises from its prospects as a means of dating Middle and Late Pleistocene bone that contains significant amounts of calcium and that is found at many sites of paleoanthropological interest. The ability to provide an isotopic method of assigning age directly to bone samples in this age range would help clarify uncertainties about chronological relationships among important fossil hominids for a time period during which major events in hominid biological and cultural evolution occurred. However, the natural ^{41}Ca/Ca ratio in terrestrial samples is expected to be very low, on the order of 10^{-14} or less. (Throughout this report the term "^{41}Ca/Ca ratio" signifies the isotopic abundance of ^{41}Ca in total calcium.) The long half-life of ^{41}Ca and its decay by electron capture to the ground state of ^{41}K (with only soft x-rays and Auger electrons with energies of about 3 keV as detectable radiation) make decay counting infeasible. We have therefore used the highly sensitive method of accelerator mass spectrometry (AMS) in an attempt to detect ^{41}Ca at natural levels. We pre-enriched the samples with an isotope separator to measure for the first time ^{41}Ca/Ca in natural terrestrial samples (*5*). For contemporary bovine bone we measured a value of ^{41}Ca/Ca of $(2.0 \pm 0.5) \times 10^{-14}$. For limestone from the surface and depths of 11 m, values of 8×10^{-15} and 3×10^{-15}, respectively, were observed.

The AMS measurements were performed with the Argonne Tandem Linac Accelerator System (ATLAS). The AMS technique makes use of the fact that ions that are accelerated to sufficient kinetic energy (typically a few million electron volts per nucle-

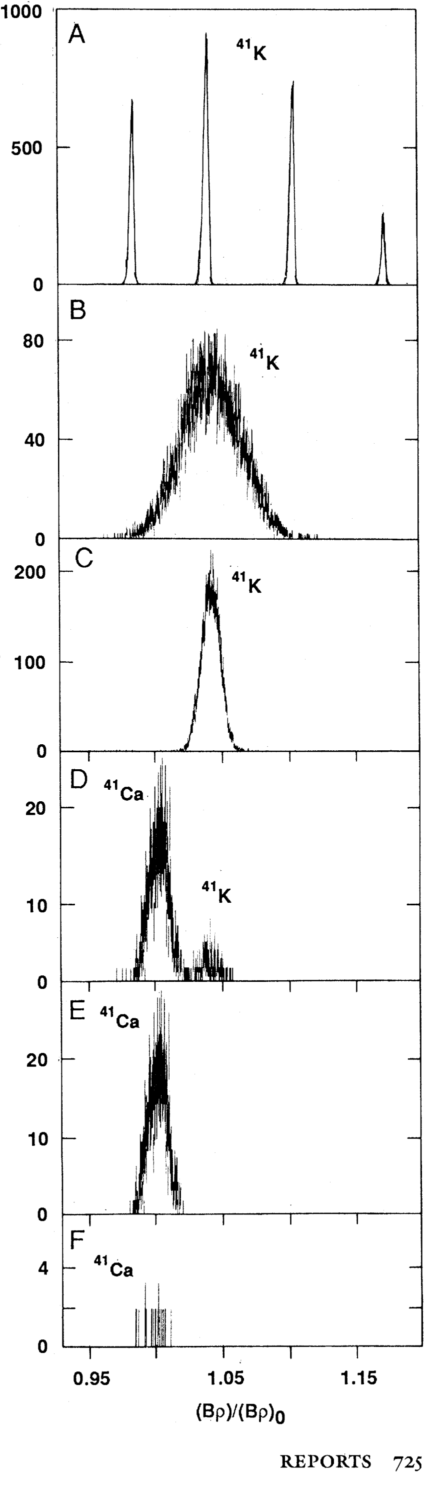

Fig. 1. Position spectra (converted to relative magnetic rigidity $B\rho$, where B is the magnetic field and ρ the bending radius) from the focal plane detector of the spectrograph for ^{41}Ca and ^{41}K yields from various sample materials are shown. The magnetically dispersed charge-state spectrum of ^{41}K ions (**A**) obtained from the passage through a thin gold foil collapsed to a single line when nitrogen gas at pressures of 1.0 torr (**B**) or 8.0 torr (**C**) was introduced into the magnetic field region. The yields for the gas-filled device at 8.0 torr from a calibration sample with known ^{41}Ca concentration are shown in (**D**) and (**E**), and for contemporary bovine (long leg) bone in (**F**). In (E) and (F) additional gating from the detector total-energy and energy-loss signals was applied. For a blank sample no ^{41}Ca counts were detected after a measuring time that was three times as long as that used for the bone sample (see text).

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on) can be unambiguously identified by nuclear charge and mass and individually counted (*6*). Metallic calcium samples were mounted in an inverted negative-ion sputter source and sprayed with NH_3 to produce $^{41}CaH_3^-$ ions for injection into the tandem. For the most abundant isotope of calcium, ^{40}Ca, a $^{40}CaH_3^-$ beam intensity of about 10 nA was produced in this way. The choice of these ions is known (*7*) to greatly reduce isobaric interference from ^{41}K, since $^{41}KH_3^-$ is not stable, at least not for lifetimes long enough for these ions to reach the tandem terminal (typically microseconds). After electron stripping and acceleration occurred in the tandem, the linac accelerated the $^{41}Ca^{10+}$ ions to a kinetic energy of 200 MeV, which is sufficient for unambiguous particle identification. In order to minimize ion losses through the system, additional electron stripping, which could have provided a higher final energy, was not used. Very efficient suppression of neighboring stable calcium isotopes was achieved through the combined filtering action of the velocity-focusing linac and the magnetic beam-transport system. We used the gas-filled magnet technique originally proposed by Cohen and Fulmer (*8*) for isobar separation. This technique is based on the fact that if charge-changing processes of an ion in a gas occur with sufficient frequency in a region of magnetic field, the trajectories will be characterized by the mean charge state $\bar{q}$ of the ion in the gas. For isobars (nuclei with the same mass number but different nuclear charge) of given kinetic energy the mean charge state $\bar{q}$ will be different. The scatter in trajectories, which determines the extent to which the two isobars can be separated, is determined at low pressure by the statistics of the charge-changing processes and at higher gas densities by small-angle scattering and energy-loss straggling (the variation in the amount of energy lost in the gas).

Table 1. Results of the pre-enrichment runs with the Calutron isotope separator. The Calutron efficiency was obtained from a detailed analysis of the collection of the stable calcium isotope ^{42}Ca. The calibration sample had a $^{41}Ca/Ca$ ratio of about 6×10^{-12} before enrichment.

Sample	Calcium vaporized in Calutron source (g)	Calutron efficiency (%)	Calcium recovered from ^{41}Ca collector (mg)	^{41}Ca enhancement
Calibration sample	77	16.6	84	152 125 ± 28*
Contemporary bovine bone	74	13.5	66	151
Limestone (surface)	71	15.2	93	116
Limestone (11-m depth)	76	13.2	85	117

*Measured with AMS (see text).

We used a broad-range Enge split-pole magnetic spectrograph that was filled with nitrogen gas to separate ^{41}Ca from the interfering stable ^{41}K isobar. Figure 1 illustrates the performance of this detection system and shows position spectra of ^{41}Ca and ^{41}K ions from various samples studied. Figure 1, A through C, shows the collapse of the magnetically dispersed charge-state spectrum of ^{41}K ions into a single line when gas is introduced into the spectrograph. Figure 1, D through F, shows (for the gas-filled device) ^{41}Ca and ^{41}K yields obtained for a calibration sample with known ^{41}Ca concentration, and a sample from contemporary bovine (long leg) bone from a cow. Limestone (nonenriched) from a depth of 11 m was used as a blank; no counts were observed for a measurement time approximately three times that for the bone. From the result for the blank sample we deduce that a $^{41}Ca/Ca$ ratio greater than 6×10^{-14} can be unambiguously determined with this system. Since pre-enriched samples were used in the present study, ratios well below this value may be determined.

Pre-enrichment by an isotope separator has been suggested (*3*) as a possible method for observing the expected low $^{41}Ca/Ca$ levels in natural samples. This is, of course, an additional (and costly) complication that one might ultimately hope to avoid by using more intense ion sources when they become available or by using accelerators with high-current positive-ion injectors (although for positive ion sources the lack of discrimination between different elemental ions will make the ^{41}K isobaric interference much more severe). The pre-enrichment also requires initial sample sizes of several hundred grams in contrast to the milligram sample sizes usually required for direct AMS measurements. However, for these initial experiments in which we attempted to establish the yet unknown ^{41}Ca concentration level in natural samples, pre-enrichment was extremely useful.

A Calutron isotope separator at Oak Ridge National Laboratory was used in the present study for the pre-enrichment of four samples of known origin. The samples consisted of (i) reactor-irradiated calcium metal with a $^{41}Ca/Ca$ ratio of about 6×10^{-12} as a check and calibration of the enrichment procedure, (ii) Illinois bovine bone, and (iii) surface (< 0.3 m) and deep rock (11 m) limestone ($CaCO_3$) from the Grantsville limestone quarry in Utah. The bovine bone was ashed and converted to $CaCO_3$. The $CaCO_3$ from the bone and from the limestone, typically 300 g per sample, was then reduced to calcium metal by a technique developed at Oak Ridge National Laboratory that uses zirconium metal as the reducing agent. More details of the sample preparation are given in (*9*). Each final metallic sample weighed approximately 75 g.

These metal samples were used as feed material for the Calutron ion source, which consumes about 100 g of calcium metal in 100 hours. The overall efficiency of the Calutron from source to collector was about 15% for the various samples. The focal-plane isotope collector consisted of an array of deep graphite pockets to minimize resputtering losses. Mass fractionation due to separator transmission and resputtering was calculated from the stable isotope yields and found to amount to less than 10% for ^{41}Ca. The calcium material from the various products was mechanically and chemically extracted and converted to CaO. Most of the material (>99%) in the ^{41}Ca pocket was ^{40}Ca. The ^{41}Ca enrichment was directly computed from the ^{40}Ca weights in the mass-40 and mass-41 pockets. The only assumption was that essentially all of the ^{41}Ca transmitted to the focal plane was

Table 2. Summary of the results of ^{41}Ca abundances. The values measured with AMS were divided by the pre-enrichment factor to obtain the original ^{41}Ca abundances.

Sample	$^{41}Ca/Ca$ ratio measured with AMS	Pre-enrichment factor	Original $^{41}Ca/Ca$ ratio
Calibration sample (nonenriched)	$(4.4 \pm 1.0) \times 10^{-12}$	1	$(4.4 \pm 1.2) \times 10^{-12}$
Calibration sample (pre-enriched)	$(5.2 \pm 0.2) \times 10^{-10}$	152	$(3.4 \pm 0.5) \times 10^{-12}$
Contemporary bovine bone	$(3.0 \pm 0.6) \times 10^{-12}$	151	$(2.0 \pm 0.5) \times 10^{-14}$
Limestone (surface)	$(8.8 \pm 4.4) \times 10^{-13}$	116	$(7.6 \pm 4.5) \times 10^{-15}$
Limestone (11 m depth)	$(4.0 \pm 1.9) \times 10^{-13}$	117	$(3.4 \pm 2.1) \times 10^{-15}$
Limestone (11 m depth) (nonenriched)	$\leq 5.8 \times 10^{-14}$	1	(3.4×10^{-15})

deposited in the ^{41}Ca pocket. We checked this assumption by comparing the efficiencies of the stable isotopes. The various parameters for the Calutron enrichment runs are listed in Table 1.

We checked the enrichment procedure by determining the ^{41}Ca/Ca ratios with AMS for the calibration samples, both with and without the Calutron pre-enrichment. The pre-enrichment factor of 125 ± 28 measured by AMS (the error stems from counting statistics only) was not significantly different from the value of 152 in Table 1. We believe, therefore, that at present the pre-enrichment factors in Table 1 are accurate to about ±15%.

The ^{41}Ca/Ca ratios in the original sample materials can now be deduced from the measured values. The results are summarized in Table 2. The quoted errors in the second column are from counting statistics only and reflect the total number of ^{41}Ca ions counted for each sample listed. The systematic uncertainty of the AMS measurements is at present estimated to be about ±15%, based on similar measurements of other radioisotopes made with the same AMS system. The errors in the last column of Table 2 include both the counting statistics and the systematic error.

First, in considering the observed concentrations, we find that the pre-enriched bovine bone and limestone samples show ^{41}Ca/Ca ratios well above the limit observed with the blank sample. This excludes instrumental effects and cross contamination in the ion source from the calibration sample, as the origin of the observed ^{41}Ca. Second, by considering the deduced original ^{41}Ca abundances, we measured a ^{41}Ca/Ca ratio for bone of $(2.0 \pm 0.5) \times 10^{-14}$. This is close to the range estimated from thermal neutron fluxes at the surface of the earth (*10*) but comfortably at the upper limit, which is encouraging for future applications. Third, the limestone samples show, within error, lower concentrations by factors of 3 and 6 for the surface and deeper location, respectively. A possible explanation for the fact that the surface limestone has a lower concentration than the contemporary bovine bone is that the limestone was covered for part of the last 100,000 years and has only been recently exposed to the surface by erosion.

Although the relatively high ^{41}Ca abundance that we have found in contemporary bone is encouraging, this by no means assures that ^{41}Ca can be used as a dating tool. ^{41}Ca, like ^{14}C, is produced by cosmic-ray neutron secondaries. However, the bulk of the isotope is made not in the atmosphere, as is the case with ^{14}C, but rather in the upper meter of the soil profile by neutron capture by ^{40}Ca. After ^{41}Ca is produced, it would be mixed with the other naturally occurring calcium isotopes into the soil matrix through ground water action. Calcium is taken up into the plant tissue in the form of Ca^{2+} through ion absorption into the root system. Radiocalcium, like all the other isotopes of calcium, would be incorporated into bone mineral through ingestion of plant materials and water. The fact that lithospheric rather than atmospheric production predominates raises the strong possibility that localized mixing and erosional effects may cause significant variations in initial ^{41}Ca/Ca ratios in many environments. In addition, since samples that have not been buried deeply enough will continue to be subject to ^{41}Ca formation, the burial histories of samples may affect ^{41}Ca concentrations. Also, one might reasonably expect postdepositional exchange of calcium in some samples through ground water contact. These and other diagenetic factors suggest that samples from each locality could exhibit unique initial ^{41}Ca concentrations. Careful additional systematic studies are necessary to answer these questions.

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