ments the temperature inside this chamber was controlled to within 0.1°C by a Lauda-Brinkmann K-4/R temperature bath and a Second Nature "Whisper 800" air circulator. Sublimation was minimized by placing crushed ice in the test chamber and thus saturating the air surrounding the apparatus.

Deformation was measured with a dial micrometer accurate to 2.54 μm and temperature was measured with a mercury thermometer, which could be read to ±0.02°C. During a test, the dial micrometer and thermometer were read at 0.5- to 2-hour intervals during the day. There was generally a gap of 6 to 7 hours in readings between about midnight and 7:00 a.m. Least-squares methods were used to calculate the creep rate over intervals of 4 to 12 hours. All tests were of sufficient duration that the creep rate decreased through a minimum value and then began to increase (Fig. 1). Total strain for the tests ranged from 0.7 to 10.7 percent.

Thin sections were cut from samples both before and after testing. Average crystal size was measured from enlarged thin-section photographs by the maximum chord-intercept method of Krumbein (*14*), a highly efficient technique for estimating the mean and standard deviation of grain sizes from thin sections when the texture is isotropic (*15*). Orientations of the *c* axis were measured on a Rigsby universal stage by standard techniques (*16*, *17*), and data were plotted in equal-area stereographic projection. Fabric strength or intensity was determined by the method of Kamb (*18*), where the intensity, f, is a measure of the deviation of the fabric data from a random distribution. The higher the value of f, the greater the degree of fabric development.

Results of these experiments indicate that the time necessary for samples to reach the minimum strain rate is a weak inverse function of crystal size (Fig. 2A). The slope of the least-squares line through the data in Fig. 2A is −1.07 (correlation coefficient, .56). Hence, doubling the average crystal size will approximately halve the time required to achieve the minimum strain rate. Jacka (*11*) found a similar relation in uniaxial-compression tests on laboratory-prepared ice; however, in Jacka's experiments the time required to achieve the minimum strain rate was almost four times longer than that required for comparable samples tested at similar temperatures and stresses in simple shear. Results also indicate that the creep of polycrystalline ice, at a relatively low stress and high homologous temperature, is sensitive to variations in both crystal size and crystal fabric (Fig. 2B). If the data presented in Fig. 2B are subjected to a multiple-regression analysis, the results can be described by the empirical relation

$$\dot{\gamma}_{min} = Bd^l f^m \tau^n \exp(-Q/RT) \quad (2)$$

where $\dot{\gamma}_{min}$ is the minimum octahedral shear-strain rate, B is a new constant, d is the average crystal size, and the other terms are as defined previously. The analysis yields $l = 3.14$, $m = 0.98$, and $B = 3.178 \times 10^{12}$ mm^{-l} bar^{-n} year^{-1} for $n = 3.0$ and $Q = 18.8$ kcal mole^{-1} with a correlation coefficient of .95. Equation 2 is plotted in Fig. 2B for an octahedral shear stress of 1.22 bars, an absolute temperature of 263.06 K, and grain sizes of 2.0, 3.0, 4.0, and 5.0 mm. The dependence of minimum strain rate on crystal size and fabric intensity is apparent from Fig. 2B. For instance, at a constant crystal size, a change in the degree of fabric development from an isotropic fabric ($f = 5$ to 7) to a strong single-maximum fabric ($f = 28$ to 30) results in about a factor of 4 increase in the minimum strain rate, in close agreement with the experimental results of Russell-Head and Budd (*4*). In addition, as crystal fabric remains unchanged, a doubling of the average crystal size results in about a factor of 9 increase in minimum strain rate. This is somewhat higher ($d^{3.14}$ versus $d^{2.50}$) than the grain size dependence observed in compression tests on laboratory-prepared isotropic samples (*9*).

ROBERT W. BAKER
Department of Plant and Earth Science, University of Wisconsin–River Falls, River Falls 54022

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Ruminant Methane $\delta(^{13}C/^{12}C)$ Values: Relation to Atmospheric Methane

Abstract. *The $\delta(^{13}C/^{12}C)$ − values of methane produced by fistulated steers, dairy cattle, and wethers, and dairy and beef cattle herds show a bimodal distribution that appears to be correlated with the plant type (C_3 or C_4, that is, producing either a three- or a four-carbon acid in the first step of photosynthesis) consumed by the animals. These results indicate that cattle and sheep, on a global basis, release methane with an average $\delta(^{13}C/^{12}C)$ value of −60 and −63 per mil, respectively. Together they are a source of atmospheric methane whose $\delta(^{13}C/^{12}C)$ is similar to published values for marsh gas and cannot explain the 20 per mil higher values for atmospheric methane.*

I have measured the $\delta(^{13}C/^{12}C)$ (*1*) of methane in gas samples taken from the rumens of five steers, two wethers, two dairy cattle, and from barns housing either beef or dairy cattle. The results show that differences in the carbon isotope ratios of the feed plants are reflected in the carbon isotope ratios of both the rumen CH_4 and the eructed CH_4. Furthermore, conclusions may be drawn about global sources of tropospheric CH_4 by comparing the data of this study with published information about both the relative magnitudes of, and carbon isotope ratios in, other known sources of atmospheric CH_4.

Rumen gas samples were collected from fistulated animals by two different methods. In the first method, a No. 18 hypodermic needle connected by a rubber hose to an evacuated 2-liter glass flask was pushed through the rubber diaphragm of the fistula in the animal's side and the flask's stopcock was opened. The flask was allowed to fill until the hissing stopped or until the animal objected and moved away. Some rumen fluid was also inadvertently collected. It

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took approximately 5 hours to transport the samples from the collection site to the laboratory where they were immediately stored at 4°C in order to inhibit any further bacterial action.

In the second method (used 10 months later), the fistula diaphragm was removed and the stopcock of an evacuated 2-liter flask was inserted directly into the rumen. The stopcock was then opened and the flask filled to atmospheric pressure. These samples were kept at room temperature since no fluid was present.

By means of the following procedure, the CH_4 in a rumen sample was separated from all other carbon compounds and quantitatively converted to CO_2 for precision isotopic analysis. A portion was mixed with purified tank O_2, and then passed through a liquid nitrogen (LN_2) trap to remove water vapor, CO_2, and any other condensables. The sample was then passed through Schutze reagent (I_2O_5 on granular silica gel) and another LN_2 trap to remove the CO that had been quantitatively converted to CO_2. The remaining mixture of O_2 and CH_4 was burned to H_2O and CO_2 by passage through an electrically heated (800°C) quartz tube containing wire-form cupric oxide and three 1-cm layers of 5 percent platinized asbestos. The CO_2 was purified of H_2O by distilling the CO_2 and H_2O mixture from a dry ice and methyl alcohol trap to an LN_2-cooled trap.

The dairy barn samples were collected with an evacuated 34-liter stainless steel cylinder. After a cow had been in its barn for 12 hours and the dairy herd in its barn for 45 minutes, a cylinder was brought in, opened, and allowed to fill to atmospheric pressure. Two air samples were collected from opposite ends of a three-sided metal shed housing 300 beef cattle. The collection vessels were a 34.4-liter stainless steel cylinder and a 97.6-liter aluminum cylinder. All the cylinders were processed for their CH_4 fraction in the same way as the rumen gas samples except that no O_2 was added.

The carbon isotopic analyses were done with a Consolidated-Nier isotope ratio mass spectrometer equipped with a semiautomatic data collecting system. The usual corrections were made for inlet fractionation, ^{17}O interferences, and spectrometer memory.

The results are grouped in Table 1 according to the major plant type [C_3 or C_4, see (*2*)] in the particular ration being fed to the animals. Carbon in plants using the C_3, or Calvin, metabolic pathway has a $\delta(^{13}C/^{12}C)$ value near -27 per mil (*3*); specifically, the published values are: for soybean (*Glycine* or *Soja*), -27.2 per mil (*4*); for alfalfa (*Medicago sativa*), -27.9 per mil (*4*); for some bromegrass species, -28 per mil (*5*); and for some timothy grass species, -28 per mil (*4*). In contrast, the carbon from plants utilizing the C_4, or Hatch-Slack, metabolic pathway has a $\delta(^{13}C/^{12}C)$ near -13 per mil (*3, 5*); the published values for corn (*Zea mays*) range from -12.6 per mil (*4*) to -14 per mil (*6*). Thus there is a difference of approximately 15 per mil in the carbon isotope ratios of the two types of feed plants.

The CH_4 samples from individual cattle eating C_3, or mostly C_3, plants had an average $\delta(^{13}C/^{12}C)$ value of -63.7 ± 7.0 per mil, whereas CH_4 samples from individual cattle eating primarily corn (C_4) had an average value of -50.3 ± 2.7 per mil. The CH_4 from a dairy herd of 40 cows eating mostly alfalfa (C_3) had a $\delta(^{13}C/^{12}C)$ value of -60.4 per mil, whereas the CH_4 from 300 head of beef cattle eating mostly corn had a value of -45.4 per mil. The CH_4 in rumen samples taken from two wethers eating alfalfa had $\delta(^{13}C/^{12}C)$ values of -70.2 and -67.0 per mil, placing them in the lighter range of the cattle eating C_3 rations. There is no clear explanation why the CH_4 in one steer rumen sample was so much lighter (-76 per mil) than the others.

The fractionation of the carbon from its original $\delta(^{13}C/^{12}C)$ value in the feed to the value found in the CH_4 is the result of digestive and bacterial actions within the rumen. Since several types of bacteria contribute to the conversion of feed to CH_4, the mechanism of the fractionation is unclear. However, carbon isotopic fractionations of this magnitude (0.97 to 0.96) caused by bacteria are not unusual (*7*). The difference in the carbon isotopic ratios between the CH_4 from the cattle fed mostly corn and the cattle fed mostly C_3 rations is caused by the difference in the carbon isotopic ratios of the original feed and is not unprecedented (*8*).

The major cattle grazing areas of the North Temperate Zone (NTZ) are in the western United States and western Siberia (*9*). There is also nomadic herding in Siberia, Mongolia, western China, Iran, Pakistan, and North Africa. European cattle feed mostly on pastures. Since the plant life of the NTZ uses, almost exclusively, the C_3 metabolic pathway (*10*), the CH_4 produced by NTZ cattle should have a $\delta(^{13}C/^{12}C)$ value near

Table 1. Carbon isotopic analyses of ruminant CH_4. All weights are wet weights or as fed.

Diet	Animal	CH_4 $\delta(^{13}C/^{12}C)$	Collection date
All (or mostly) C_3 plants			
Timothy and bromegrasses	Milk cow in barn (air sample)*	−61.6	23 July 1979
Alfalfa (13 lb/day); ground soybeans plus corn (4 lb/day); and corn silage (4 lb/day)	Steer (rumen sample)†	−61.8	31 July 1979
Alfalfa	Steer (rumen sample)	−61.1	31 July 1979
Alfalfa (15 lb/day); ground corn (4 lb/day); and soybeans (1 lb/day)	Steer (rumen sample)‡	−58.1	16 May 1980
Alfalfa (15 lb/day); ground corn (4 lb/day); and soybeans (1 lb/day)	Steer (rumen sample)‡	−76.0	16 May 1980
Average		−63.7 ± 7.0	
Alfalfa	Dairy herd of 40 animals (air sample)§	−61.1	28 July 1980
Alfalfa	Wether (rumen sample)	−70.2	31 July 1980
Alfalfa	Wether (rumen sample)	−67.0	16 May 1980
Mostly corn (a C_4 plant)			
Corn silage (10 lb/day); and ground corn and soybeans (14 lb/day)	Steer (rumen sample)†	−50.6	31 July 1979
Corn silage (32 lb/day); ground corn (36.8 lb/day); and soybeans (9.2 lb/day)	Dairy cow (rumen sample)‡	−52.8	16 May 1980
Corn silage (30 lb/day); ground corn (32 lb/day); and soybeans (8 lb/day)	Dairy cow (rumen sample)‡	−47.4	16 May 1980
Average		−50.3 ± 2.7	
Mostly ground corn	Beef herd of 300 animals (air sample)‖	−45.4¶	16 May 1980

*The CH_4 concentration in the air (by volume was 47 parts per million (ppm). †The two animals were housed together. ‡These four animals were housed together. §The CH_4 concentration in the air (by volume) was 39 ppm. ‖In an upwind corner of barn the CH_4 concentration in the air (by volume) was 6.35 ppm, and in a downwind corner, 9.73 ppm. ¶Average of upwind (− 45.3 per mil) and downwind (− 45.4 per mil) $\delta(^{13}C/^{12}C)$ of CH_4.

that for the cattle eating C_3 rations reported in this study. However, many cattle in the NTZ receive food rations of corn and sorghum, both C_4 plants. For instance, approximately 25 percent of a steer's diet in the United States is corn and sorghum (*11*). For Europe and the Soviet Union, the amount is less, only about 10 percent. Nomadic herds do not get special rations. The total corn and sorghum fed in the United States, the Soviet Union, and Europe, if distributed throughout the entire cattle population of the NTZ (660×10^6) (*12*), would be equivalent to 8 percent of the total bovine diet. Livestock fed on diets containing a significant amount of grain produce perhaps 10 percent less CH_4 than livestock eating only hay or alfalfa (*13*). However, for the present study such a difference is immaterial. Thus, carbon in the CH_4 from NTZ cattle should be perhaps 1 per mil heavier than the carbon in CH_4 from cattle eating only C_3 plants.

Another 560×10^6 cattle live in the tropics and South Temperate Zone (STZ) (*12*). Although the distribution of C_3 and C_4 plants in their diet is not known, many of the forage crops and pasture grasses they eat use the C_4 metabolic pathway (*10*). Thus, the CH_4 from these cattle should have a $\delta(^{13}C/^{12}C)$ value similar to, or lighter than, the CH_4 from corn-fed cattle.

Sheep, the only other significant source of ruminant methane, produce about 6 percent as much CH_4 as cattle (*12, 14*). Their geographic distribution is approximately the same as that of cattle (570×10^6 in the NTZ and 470×10^6 in the tropics and STZ) (*12*) and their grazing diet is similar. Only the isotopic composition of CH_4 from two wethers fed C_3 plants was measured in Illinois (−70.2 per mil and −67.0 per mil, respectively). If sheep eating C_4 plants give off CH_4 that is 14 per mil heavier in ^{13}C (as in the case of cattle), the worldwide average $\delta(^{13}C/^{12}C)$ value for ovine methane would be −63 per mil or lighter. [It is interesting that the methane released by marshes and lakes, apparently a very different environment, has an average $\delta(^{13}C/^{12}C)$ of −65 per mil (*15*).]

The published value for the average $\delta(^{13}C/^{12}C)$ of atmospheric CH_4 is −41 per mil (*15*), and the measured carbon kinetic isotope effect in the reaction of CH_4 with OH, its principal tropospheric sink, is 1.003 (*16*). Therefore, the calculated average $\delta(^{13}C/^{12}C)$ value for all sources of tropospheric CH_4 is −44 per mil. None of the ruminant CH_4 carbon isotopic ratios reported here match the calculated source average. Evidently, then, ruminant CH_4 is quite different isotopically from the average source of atmospheric CH_4.

According to Ehhalt and Schmidt (*14*) and Baker-Blocker *et al.* (*17*), cattle and sheep combined generate 1×10^{14} g of CH_4 per year (at least 67 percent of all herbivorous production), which is 10 to 20 percent of the annual global release of CH_4 to the atmosphere. Consequently, other significant sources of CH_4 enriched in ^{13}C compared to ruminant CH_4 account for the average $\delta(^{13}C/^{12}C)$ of all sources. Such sources are unknown (*14*).

FLEET RUST*

Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439

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* Present address: 6090 Los Angeles Avenue, No. 198, Simi Valley, Calif. 93063

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Korean Hemorrhagic Fever: Propagation of the Etiologic Agent in a Cell Line of Human Origin

Abstract. *The etiologic agent of Korean hemorrhagic fever has been propagated in a human cultured cell line derived from a carcinoma of the lung. The cells, described as type II, alveolar epithelial, support replication of the agent and successive passages. Antigen of the Korean hemorrhagic fever agent is readily detected in infected cells by means of direct or indirect fluorescent antibody techniques. Previous attempts to propagate this agent in vitro had been unsuccessful.*

Korean hemorrhagic fever (KHF), which is presumed to be of viral origin, is one member of a group of similar hemorrhagic fevers with renal syndrome that occur throughout large portions of the world from Japan in the East, throughout Soviet Russia, to Sweden in the West (*1–4*).

H. W. Lee and co-workers reported recently the isolation of the KHF etiologic agent from a rodent, *Apodemus agrarius coreae* (*1*). Specific antigen for KHF was detected in various tissues of this rodent, by using an indirect fluorescent antibody (IFA) technique (*5*) and samples of serum from human patients recovering from KHF. Isolation of the KHF agent had been attempted by several groups of investigators since 1952 (*1, 2, 6–8*). Attempts to propagate the agent in cell culture have also been made, without success (*1, 3, 8, 9*). However, we now report the successful propagation of the etiologic agent of KHF in an in vitro substrate, a human cell line. This line, designated A-549 and described as type II, alveolar epithelial cells, was derived from a carcinoma of the lung (*10, 11*).

The starting infectious material for this study was a pooled suspension (10 percent) of lung tissue from several *A. a. coreae* killed 21 days after they were inoculated with pooled tissue from several *Apodemus* infected with fourth-passage KHF strain 76-118 (*1*). A 10^{-1} dilution of this material in medium E-199 containing 10 percent fetal calf serum (FCS) was inoculated onto monolayers of various primary and continuous avian and mammalian cells prepared on 12-mm glass cover slips in 24-well plates (*12*). The cover slip monolayers were maintained with medium E-199 with 5 percent FCS which was changed at 3- to 5-day intervals as required. Inoculated cultures were examined daily for cytopathogenic effect (CPE). On day 8 after inoculation and at 2-day intervals thereafter through day 24, two to three cover slips were removed, fixed in 100 percent cold acetone, and examined by the IFA tech-

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