A mass-15 peak is also observed, presumably due to $N^{15}+$. The heavy isotopes of nitrogen and oxygen are found in the ionic state in the upper atmosphere in abundance ratios equal to or less than those at the earth's surface.

The mass-20 peak is thought to be due to neon. The abundance of neon at the earth's surface is three times that of helium. At 900 km the $20^+/He^+$ ratio was observed to be of the order of 1 percent.

Near apogee, the mass spectra exhibit peaks only at mass 1, 2, 4, and, at times, 8 amu. Again, these peaks presumably are due to ions of hydrogen, deuterium, helium, and $O++$ or He^+_2. The mass-18 peak was observed early in the life of the satellite, but was not roll-modulated and gradually disappeared with time as the outgassing of the satellite diminished. The predominant ion is H^+. The D^+/H^+ ratio is less than 2 parts in 10^4, and the He^+ abundance is a few tenths of 1 percent. The mass-8 peak appears in this altitude region where there is no mass 16 (O^+). Its abundance is of the order of that of the mass-2 ion.

The experiment described is still operating normally (September 1966).

JOHN H. HOFFMAN*
E. O. Hulburt Center for Space Research, U.S. Naval Research Laboratory, Washington, D.C. 20390

Notes

1. Supported by a grant from NASA, S-60376-G.
* Present address: Southwest Center for Advanced Studies, Dallas, Texas.

28 October 1966

Deuterium Isotope Effect on Carbon Isotope Fractionation in Photosynthesis

Abstract. *Plants grown in D_2O show a decreased tendency to fractionate carbon-13 during photosynthetic incorporation of carbon dioxide. The isotopic ratio C^{13}/C^{12} of the tissues of deuterated plants appears to be proportional to the deuterium content of the tissue. This effect was found in specimens of the partially deuterated vascular plant* Nicotiana tabacum *as well as in cultures of the fully deuterated alga* Chlorella vulgaris.

Slight variations in the carbon isotope ratio of plant material were first noted by Nier and Gulbranson (*1*). Extensive compilations made by Wickman (*2*) and Craig (*3*) of the C^{13}/C^{12} ratio in plant tissue show that all plants discriminate to the extent of a few percent against the heavy isotope of carbon during photosynthetic carbon dioxide fixation. Furthermore, different plant types within the various taxonomic groups have C^{13}/C^{12} ratios that fall within rather narrow ranges. After the initial fixation of carbon dioxide, subsequent fractionations may occur in various metabolic reactions and syntheses, and some of these have been studied by Abelson and Hoering (*4*) and by Park and Epstein (*5*, *6*). Since deuterated plants have been found to show significant differences in morphology and biochemistry as compared to the same species of normal isotopic composition (*7*), it is not surprising that the patterns of isotopic fractionation may be altered in the isotopically altered organism.

Seeds of field-grown tobacco plants (*Nicotiana tabacum* L.) were germinated in water, then transferred to deuterated media when the seedlings were several centimeters high (*8*). Growth took place in well-aerated, hydroponic solutions containing only carbon-free, inorganic salts and up to 70 percent D_2O in the culture media. After growth and onset of inflorescence, stems, flowers, seed parts, and leaves of various ages were desiccated in vacuum and burned in a closed system that permitted collection of the water of combustion and carbon dioxide. The deuterium fixed in the tissue was determined by infrared analysis of the collected water (*9*), and the carbon-isotope ratio was determined mass spectrometrically. Isotope ratios were determined by comparison with a local standard, then related to the Solenhofen limestone standard by a correction factor. The upper group of points in Fig. 1 shows the carbon isotope ratios for tobacco, plotted in units of δ per mil as a function of the deuterium content. The value of δ (per mil) is defined as

$$\delta = \left(\frac{C^{13}/C^{12}\ (\text{sample})}{C^{13}/C^{12}\ (\text{standard})} - 1 \right) \times 10^3$$

The error in the determination of δ is estimated as $\pm$ 0.1 per mil.

The curve drawn in Fig. 1 is based on a least-squares analysis of all the points, a linear relation between deuterium content and δ value being assumed. Extrapolation of the curve to zero deuterium concentration yielded a value for the intercept of $\delta = -28$. This value is within the range found for isotopically normal vascular plants growing in atmospheric carbon dioxide, and compares well with the δ value determined for the water-grown control plants, $\delta = -28.5$. We believe the indicated slope from the least-squares analysis is real, and this is confirmed by experiments on algae, described below, in which the variables were better controlled. It should be emphasized that the scatter is a result of biological variability and is not due to analytical errors in the mass spectrometry.

The spread of the points for tobacco may be due to the grossness of the sampling technique employed and lack of control over important variables. A typical combustion sample of stem or leaf represented tissue formed during a large fraction of the lifetime of the plant, during which the deuterium content, as well as fixed-carbon content of the tissue, was continually changing in an unknown manner. Also, the isotopic makeup of material being translocated was unknown; cytoplasmic carbon dioxide has been shown to have a carbon-isotopic ratio significantly different from from that of other plant parts (*5*). Moreover, it was not possible in the experiment to correct for the carbon isotopic ratio present in tissue before transplantation of seedlings to deuter-

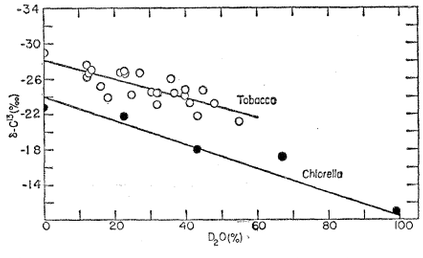

Fig. 1. Fractionation of carbon isotopes in *N. tabacum* and in *C. vulgaris* as a function of the deuterium level of the tissues. The δ values for *C. vulgaris* have been corrected to express enrichment relative to the input carbon dioxide (measured value for δ_{CO_2} in feed gas was —25.3 per mil). Tobacco plants were grown in D_2O concentrations of 0, 30, 50, 60, and 70 percent D_2O; *Chlorella* cultures were grown in deuterium concentrations of 0, 25, 50, 75, and 99.6 percent D_2O.

ated media, although analysis showed that leaves formed late in the lifetime of the plant were more highly deuterated than older leaves. As a test plant, tobacco suffered from the disadvantage that the range of available deuterium concentrations is limited, no higher plant having yet been grown in a completely deuterated medium (*10*). For these reasons, a more closely controlled experiment was devised, employing unicellular algae adapted to life in pure D_2O.

Completely deuterated *Chlorella vulgaris,* which had been mass cultured in heavy water for several years (*11*), was used to inoculate media of 75 percent and 99.6 percent D_2O composition; the hydrogen form of the same strain was used to inoculate media of 0, 25, and 50 percent D_2O concentrations. Flask cultures with media of these concentrations were grown on a shaker with conditions of illumination, gas flow, and agitation made as identical as possible for all cultures, as it has been shown that the conditions of growth of an algal culture may significantly change the apparent carbon isotopic fractionation factor (*4*). Cultures were aerated with a gas mixture composed of 95 percent nitrogen and 5 percent carbon dioxide. Under the wide range of deuterium concentrations used, the growth rates for the various cultures were widely different. Therefore, cultures were harvested, not after a specified time, but when an arbitrary cell density had been attained [$(3 \pm 0.5) \times 10^8$ cells/ml]. Growth times varied from 22 to 27 days.

After harvest, the cells were burned and the deuterium content and carbon isotopic ratios were determined. The deuterium content of the lyophilized cells did not correspond to the concentrations of deuterium in the media, owing to isotopic fractionation during metabolism. The lower group of points in Fig. 1 shows the results of the *Chlorella* experiments. The δ values in this case indicate enrichment relative to the initial isotopic content of the feed gas. The enrichment shown by the H_2O control culture was $\delta = -22.88$ per mil, in good agreement with the value of $\delta = -25.8$ per mil reported by Abelson and Hoering (*4*) for the closely related organism *Chlorella pyrenoidosa* under similar growth conditions.

That both curves of Fig. 1 have about the same slope suggests that a similar mechanism may be responsible in both cases for the observed isotopic fractionations. The observed δ values for tobacco, while covering only slightly more than one-half the range of deuterium concentrations, show a variation equal to that found throughout the range of plant species, as tabulated by Craig (*3*).

In our opinion the change in fractionation shown by deuterated organisms is most likely a consequence of widespread changes in the cell, and the present data do not allow interpretation in terms of kinetic isotope effects on specific chemical reactions.

ROBERT A. UPHAUS
JOSEPH J. KATZ
Argonne National Laboratory,
9700 South Cass Avenue,
Argonne, Illinois 60439

References and Notes

1. A. O. Nier and E. A. Gulbranson, *J. Am. Chem. Soc.* **61**, 697 (1939).
2. F. E. Wickman, *Geochim. Cosmochim. Acta* **2**, 243 (1952).
3. H. Craig, *ibid.* **3**, 53 (1953).
4. P. H. Abelson and T. C. Hoering, *Proc. Natl. Acad. Sci. U.S.* **47**, 623 (1961).
5. R. Park and S. Epstein, *Geochim. Cosmochim. Acta* **21**, 110 (1960).
6. ———, *Plant Physiol.* **36**, 133 (1961).
7. E. Flaumenhaft *et al., Intern. Rev. Cytol.* **18**, 313 (1965); J. J. Katz and H. L. Crespi, *Science* **151**, 1187 (1966).
8. Studies on tobacco were undertaken as part of a larger program in collaboration with M. I. Blake and F. T. Crane of the departments of Pharmacy and Pharmacognosy, University of Illinois, Chicago.
9. H. L. Crespi and J. J. Katz, *Anal. Biochem.* **2**, 274 (1961).
10. R. A. Uphaus *et al., J. Pharm. Sci.* **54**, 202 (1965); M. I. Blake, F. A. Crane, R. A. Uphaus, J. J. Katz, *Lloydia* **27**, 254 (1964).
11. H. F. DaBoll, H. L. Crespi, J. J. Katz, *Biotechnol. Bioeng.* **4**, 281 (1962).
12. Work performed under the auspices of the AEC. We thank O. Van Hook for mass spectrometer determinations.

3 October 1966

Fractionation of Potassium/Rubidium by Amphiboles: Implications Regarding Mantle Composition

Abstract. *We show that the rubidium in amphiboles is generally depleted with respect to potassium. The K:Rb ratios of 50 analyzed amphiboles range from 100 to 5000, averaging 1120. This fractionation effect holds for potassium concentrations ranging from 0.05 to 1.5 percent. The K:Rb ratios of abyssal tholeiites do not place unambiguous limits on the K:Rb ratio of the upper mantle, since partial melting of a mantle material such as amphibole peridotite would produce a liquid with a K:Rb ratio higher than that in the initial material. Large-scale mineralogic control of distributions of trace elements in the mantle could produce trends with depth that are the reverse of trends normally attributed to differentiation processes.*

Interest in use of the K:Rb ratio as a geochemical tracer was revived by the demonstration (*1*) that the K:Rb ratio of terrestrial rocks is not invariant but may vary significantly as a function of geologic process. Many papers have since discussed variations in the K:Rb ratio of rocks and their possible interpretation (*2–5*). Perhaps the most interesting was the finding (*2*) that oceanic abyssal basalts exhibit K:Rb ratios ranging from 475 to 1830, compared to the value of 240 that is believed to characterize continental igneous rocks (*6*).

We shall now discuss the role of mineralogy with respect to K/Rb fractionation. In particular, we show that amphiboles strongly discriminate against rubidium, and that amphiboles from most geologic occurrences have above-normal K:Rb ratios.

Ratios ranging from 400 to 1400 have been determined for amphibole peridotites from Saint Paul Rocks, with the amphibole content and K:Rb ratio varying sympathetically (*7*). Ratios as high as 2800 can also be derived from data published in connection with the K-Ar dating of amphiboles (*8*). While a preferential exclusion of rubidium from the amphibole structure is suggested by such data, a more convincing demonstration can be found in analyses of coexisting minerals (Table 1).

The mineral assemblages of both

Table 1. Potassium-rubidium data for coexisting phases (*9*). TQ2: metamorphic aureole, Tinaquillo, Venezuela. B65: Baltimore gneiss, Woodstock Dome, Maryland.

Mineral	K		Rb (ppm)	K/Rb
	ppm	Percent		
	TQ2 (10)			
Hornblende	465		0.196	2400
Pyroxene	89.4		.134	670
Plagioclase	274.		.972	280
	B65 (11)			
Hornblende		1.260	13.95	902
Pyroxene		0.0781	3.21	243
Plagioclase		.951	27.8	342