

cepts such as deep-tissue half-times (14), diffusion versus perfusion limitations, gas-induced fluid shifts (24), preexisting gas micronuclei (21, 22), and appropriate ascent criteria in a way that is insensitive to the experimental difficulty inherent in decompression.

BRIAN G. D'AOUST, K. H. SMITH
H. T. SWANSON, R. WHITE

Virginia Mason Research Center,
Seattle, Washington 98101

CLAUDE A. HARVEY*

WILLIAM L. HUNTER

Naval Submarine Medical Center,
Groton, Connecticut 06340

TOM S. NEUMAN, ROBERT F. GOAD
Submarine Development Group I,
San Diego, California 92132

References and Notes

- C. J. Lambertson and W. K. H. Sundmaker, in *Effect of Respiratory Gases at Extreme Pressure [Predictive Studies 3]*, C. J. Lambertson, Ed. (Report of the Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, 1972).
- G. D. Blenkarn, C. Aquadro, B. A. Hills, H. A. Saltzman, *Aerosp. Med.* **42**, 141 (1971).
- J. Idicula, D. J. Graves, J. A. Quinn, C. J. Lambertson, in (4), vol. 5.
- C. J. Lambertson, Ed., *Underwater Physiology* (Federation of American Societies for Experimental Biology, Bethesda, Md., 1976).
- C. J. Lambertson, in (4), vol. 4, p. 35.
- D. J. Graves, J. Idicula, C. J. Lambertson, J. A. Quinn, *Science* **179**, 582 (1973); R. H. Strauss and T. D. Kunkle, *ibid.* **186**, 443 (1974); C. J. Lambertson and J. Idicula, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 455 (1974); *J. Appl. Physiol.* **39**, 434 (1975).
- K. Green, Laboratory Report, Institute for Environmental Medicine, University of Pennsylvania Medical Center, Philadelphia (1975).
- The first reports identifying this phenomenon as a gas bubble lesion associated with cutaneous gas lesions and vestibular derangement in man were those of C. J. Lambertson's group (1, 2) and were presented at the Fifth Symposium on Underwater Physiology in 1972. They demonstrated that man develops cutaneous gas lesions at 37 atm absolute breathing N₂ or Ne at high ambient pressures of He. Experimental confirmation by Idicula *et al.* (3) demonstrated copious production of bubbles in anesthetized pigs under a total ambient pressure of 1 atm when N₂O was respired and the animals were surrounded by He. The severity of lesions in the latter experiment was directly proportional to the solubility of the gas respired. This result identifies solubility and therefore total gas concentration as one of the critical parameters in predicting bubble formation and is confirmed by other research [E. Hemmingsen, *Science* **167**, 1493 (1970); B. G. D'Aoust and L. S. Smith, *Comp. Biochem. Physiol.* **49**, 331 (1976); D. L. Beyer, B. G. D'Aoust, E. Casillas, L. S. Smith, paper presented at the Sixth Symposium on Underwater Physiology, San Diego, 6 to 10 July 1975.
- D. Haugen and E. Belcher, "Final report ONR N00014-69C-0402" (Applied Physics Laboratory, University of Washington, Seattle, 1976); E. O. Belcher, thesis, University of Washington (1976.) Under a surgical plane of anesthesia, a right lateral thoracotomy was made to expose the required major vessels of the heart. The Doppler ultrasonic bubble detection cuff was placed around the posterior vena cava, after which the chest wall was closed; the leads from the Doppler cuff were run subcutaneously to the lateral dorsum of the back approximately 5 cm posterior to the scapula, where they exited through a Dacron-covered storage pack (which allowed healing) with a sterile seal around the connectors. Further details are available in K. H. Smith and B. G. D'Aoust ["Final report ONR N000129-76MB-498" (Virginia Mason Research Center, Seattle, Wash., 1976)].
- The appearance of bubbles in the first 40.2-m experiment but not in two subsequent ones (either with the same pair of animals or with two more naive subjects) suggests the potential importance of a possible fright reaction to an initially high noise level associated with gas flushing. Such a reaction may have physiologically predisposed the animals to bubble production through vasoconstriction and resulting lower perfusion of slow tissues. Gas flushing in subsequent experiments was carried out in such a way as to slowly increase the onset of noise during the gas flush.
- K. H. Smith, paper presented at the Ninth Undersea Medical Society workshop, Bethesda, Md., 21 to 23 February 1975.
- The total tissue tension and fractional saturation values for He and N₂ (Fig. 1) have been computed from the equation

$$\pi_2 = fD_1 + fR(T - 1/K) - (\pi_1 - fD_1 - fR/K)e^{-KT}$$
 where T is the step time in minutes, π_1 is the initial gas tension at $T = 0$, π_2 is the final gas tension at T , D_1 is the depth at $T = 0$, R is the rate of change of pressure (dP/dT) in meters per minute, f is the inert gas decimal fraction, K (the tissue constant) is $0.693/T_{1/2}$, and $T_{1/2}$ is the tissue half-time in minutes. Saturating He tensions and desaturating N₂ tensions were calculated and summed for discrete pairs of N₂ + He half-times as follows: 13 + 5, 22.5 + 9, 37.4 + 15, 60.2 + 25, 93 + 41, 139 + 63, 200 + 94, 278 + 138, 372 + 182, 480 + 240. The rationale for this procedure has been described by Smith (11). The excess gas concentration was calculated for all tissues by assuming an average fat composition of 15 percent by weight for all tissues and by finding the difference between the excess inert gas fraction and the same gas fraction at a saturation ratio of 1.00.
- B. G. D'Aoust, paper presented at the annual meeting, Undersea Medical Society, Toronto, Ontario, 14 to 16 May 1977.
- P. B. Bennett and D. H. Elliott, Eds., *The Physiology and Medicine of Diving and Compressed Air Work* (Williams & Wilkins, Baltimore, ed. 2, 1975).
- A. R. Behnke, in (14), p. 392.
- R. D. Workman and R. C. Bormann, in (14), p. 307.
- H. V. Hempleman, in (14), p. 331.
- A. A. Buhlman, in (14), p. 348.
- Diffusion of gases in liquids is constrained chiefly by molecular dimensions, whereas relative diffusion rates of gases in the gas phase follow Graham's law. The difference is large for He but is less for other diving gases.
- A. E. Boycott, G. C. C. Damant, J. S. Haldane, *J. Hyg. Cambridge* **8**, 342 (1908); H. R. Schreiner and P. L. Kelly in (4), vol. 4, p. 209. The multi-exponential parallel compartment model expresses gas saturation and desaturation of a number of "compartments" or tissues as a function of the pressure gradient between tissue and alveolar gas and a constant $KT_{1/2}$ numerically equal to $0.693/T_{1/2}$. Usually a group of 9 to 15 tissues is used for N₂ of half-times ranging from 5 to 480 minutes.
- C. Harvey and C. J. Lambertson, in *Proceedings of the Sixth Symposium on Underwater Physiology* (San Diego, 6 to 10 July 1975), in press.
- E. N. Harvey, in *Decompression Sickness*, J. F. Fulton, Ed. (Saunders, Philadelphia, 1951), p. 90; A. Evans and D. Walder, *Nature (London)* **222**, 251 (1969).
- R. Tepper, paper presented at the annual meeting, Undersea Medical Society, Toronto, Ontario, 14 May 1977.
- B. A. Hills, *Aerosp. Med.* **42**, 664 (1971); *Clin. Sci.* **40**, 175 (1971); *Arch. Intern. Med.* **129**, 356 (1972); J. A. Kylstra, I. S. Longmiur, M. Grace, *Science* **161**, 289 (1968); B. A. Hills, *Rev. Subaquatic Physiol. Hyberbar. Med.* **2**, 3 (1970).
- B. G. D'Aoust, K. H. Smith, H. T. Swanson, *J. Appl. Physiol.* **41**, 348 (1976).
- We thank R. L. Stayton, J. Moore, M. Knowles, J. Mahoney, T. Baker, and S. Donaldson for expert technical assistance. Supported by grant N000129-76MB-498 from the Department of the Navy and by grant HL17503 from the Division of Lung Diseases of the National Heart, Lung, and Blood Institute.

27 December 1976; revised 14 February 1977

Carbon-13/Carbon-12 Ratio Is Relatively Uniform Among Honey

Abstract. *The variability of the carbon-13/carbon-12 ratio in honeys was evaluated preliminary to use of the ratio to detect the addition of high-fructose corn syrup to honey. Eighty-four honey samples representing 34 states and including 37 floral types from 17 plant families were analyzed. The mean value of the per mil increment in carbon-13 ($\delta^{13}C$) for all samples is -25.2 per mil, and the coefficient of variation is 3.7 percent. This is the smallest variation yet encountered for a honey constituent or physical property. The range and magnitude of the values suggest that the floral sources are C₃ plants.*

We have undertaken to develop methods to detect the undeclared addition to honey of high-fructose corn syrup (HFCS), a new, highly refined syrup now produced in large quantity (1). Its similarity to honey in major components and in minor oligosaccharides and the great variability of the composition of honey make most approaches unfruitful.

When inorganic carbon is converted to living matter during photosynthesis, an isotope effect produces differences in the ¹³C/¹²C ratios among the various reservoirs of the carbon cycle (2). The organic compounds of cells invariably have a slightly lower ¹³C/¹²C ratio than the carbon dioxide and carbonate of the environment. Isotope variations among these reservoirs result from differences in chemical and physical properties of molecules containing different isotopic species (3).

In surveys of many plant families,

Bender (4) and Smith and Epstein (5) demonstrated large differences in $\delta^{13}C$ values (6) among plants. Bender, in analyzing the family Gramineae, found that plants that initially fix carbon dioxide via the C₄ dicarboxylic acid pathway have $\delta^{13}C$ values in the range -10 to -20 per mil and those which follow only the C₃ cycle have $\delta^{13}C$ values of -22 to -33 per mil. Thus C₃ plants fractionate atmospheric carbon dioxide to a greater extent than do C₄ plants. Families that show crassulacean acid metabolism also have many examples of high $\delta^{13}C$ values. Smith and Epstein looked at many plant families and found several to be high in ¹³C content ($\delta^{13}C$ range, -5.6 to -18.6 per mil), while those lower in ¹³C content ($\delta^{13}C$ range, -23.2 to -34.3 per mil) comprise the bulk of the plant kingdom, lacking the C₄ pathway of carbon dioxide fixation.

Smith and Epstein (5) suggested that

Table 1. Carbon-13/carbon-12 ratios of selected U.S. honeys. Carbon isotope ratios were determined by Geochron Laboratories Division of Krueger Enterprises Inc. (Cambridge, Mass.) on an AEI MS-20 double-collecting, 180°-sector mass spectrometer with a dual-capillary inlet. Usual corrections were applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, tailing of major onto minor peak signals, and contribution of ¹⁷O to the mass 45 signal. Samples were combusted in purified O₂ at about 850°C and the gases were recirculated over CuO at 850°C for 10 minutes; water and CO₂ were frozen, excess O₂ was removed, and CO₂ was transferred to a sample flask for analysis. Results are expressed in δ¹³C units (6). The overall reproducibility for the system was stated to be 0.3 per mil in the ratio; however, five duplicate samples of honey agreed within 0.1 per mil. Interlaboratory crude oil reference samples also agreed within 0.1 per mil overall.

Family	Floral type	Number of samples	δ ¹³ C (per mil)
Anacardiaceae	<i>Schinus molle</i> (pepper tree)	1	-25.0
Aquifoliaceae	<i>Ilex glabra</i> (gallberry)	1	-25.6
Compositae	<i>Centaurea solstitialis</i> (star thistle)	1	-26.3
Cornaceae	<i>Nyssa ogeche</i> (tupelo)	1	-26.0
Cyrillaceae	<i>Cyrilla parvifolia</i> (titi)	1	-24.2
Euphorbiaceae	<i>Sapium sebiferum</i> (tallow tree)	1	-26.4
Labiatae	<i>Salvia</i> spp. (sage)	1	-24.2
Leguminosae	<i>Glycine soja</i> (soybean)	2	-26.8
Leguminosae	<i>Medicago sativa</i> (alfalfa)	10	-25.2
Leguminosae	<i>Melilotus</i> spp. (sweet clover)	2	-26.4
Leguminosae	<i>Trifolium</i> spp. (clover)	11	-25.6
Magnoliaceae	<i>Liriodendron tulipifera</i> (tulip tree)	1	-25.3
Malvaceae	<i>Gossypium hirsutum</i> (cotton)	1	-24.7
Onagraceae	<i>Epilobium angustifolium</i> (fireweed)	1	-25.4
Palmae	<i>Sabal</i> spp. (palmetto)	1	-24.7
Polygonaceae	<i>Fagopyrum esculentum</i> (buckwheat)	1	-25.2
Rosaceae	<i>Rubus</i> spp. (blackberry)	1	-26.1
Rutaceae	<i>Citrus</i> spp. (orange, grapefruit)	3	-23.4
Tamaricaceae	<i>Tamarix gallica</i> (tamarisk)	1	-25.1
Tiliaceae	<i>Tilia americana</i> (basswood)	1	-25.6
	Unclassified natural season blends	15	-25.2
	Honeydew honey	4	-24.5

one could easily distinguish whether a particular brand of sucrose was obtained from sugarcane (C₄ plant) or from sugar beet (C₃ plant), a distinction not possible using classical chemical methods. Nissenbaum *et al.* (7) used this approach to detect the addition of sucrose to Israeli citrus juice. They found the δ¹³C values of citrus juice solids (-24.3 to -25.0 per mil) to be very similar to that of beet sugar (-24.3 per mil) but very different from that of cane sugar (-12.2 per mil). Unfortunately, beet sugar is used almost exclusively in Israel so no practical application of this method was realized. Recently, however, Hillaire-Marcel *et al.* (8) utilized this method to detect the adulteration of maple syrup with cane sugar. The δ¹³C for cane sugar was -11.47 ± 0.5 per mil, for Vermont maple syrup -24.05 ± 0.45 per mil, and for Quebec maple sugar -23.54 ± 0.40 per mil. They reported the ability to detect adulteration at the 10 percent level.

Listings of δ¹³C values (4, 5) show rather wide ranges for some nectar-producing plants: two Compositae (5) have values of -24.2 and -34.3 per mil, and among five Euphorbiaceae, a range from -15.3 to -29.4 per mil is recorded. To evaluate this approach for the detection of corn products in honey, information is needed on the variability of δ¹³C among honeys of different origin.

We have collected directly from U.S. beekeepers nearly 500 certified samples of honey from the 1974 and 1975 crops. For 84 representative samples selected from these, we have obtained δ¹³C values. Samples from 34 states included 37 named floral types from 17 plant families. The criteria for sample selection were such that (i) the geographic distribution was proportional to that of honey production, (ii) for several floral types and blends produced in greatest amount, samples were selected from as wide an area as possible, (iii) at least one example of each commercially significant type was included, (iv) natural mixtures representing production for the entire season were selected from many areas, and (v) honeydew honeys, identified by positive optical rotation, were included. Table 1 summarizes the results for at least one sample from each family. The identification of floral source by the beekeeper is shown. Values for each sample with data on floral source and area of production will appear elsewhere (9). For the objective of this work, the important aspect was that the samples represent honey types commonly available in quantity. In view of the remarkable uniformity of the data, the need for unequivocal floral source identification appears secondary. For a study of the relatively small differences among honey

types and areas of production, examination of hand-collected nectar would be the obvious choice.

The mean δ¹³C for all samples was -25.2 per mil; the coefficient of variation was 3.7 percent. This is the smallest variation yet encountered for any honey constituent or physical property. An analysis of 490 samples for major components (10) showed the smallest coefficient of variation (fructose) to be 5.4 percent; most minor constituents varied between 25 and 75 percent, and for trace components a 10- to 100-fold variation was sometimes seen (11).

Even though honeybees may range for miles around the colony, with access to many nectar plants, nectar from the major sources appears to greatly dilute any contributions from high-¹³C plants. It is perhaps noteworthy that the three samples with the highest ¹³C contents (*Prosopis glandulosa*, Hawaii, -22.5; *Acacia greggii*, Arizona, -22.8; and season blend, Arizona, -22.7 per mil) originated in xeric environments, which have been suggested to favor adaptation to a high ¹³C/¹²C ratio (5). The almost exclusively carbohydrate nature of honey must also contribute greatly to the uniformity of the values. The ranges of δ¹³C values for the constituents of single plants (12) are wider than the range found here for all the samples examined. Details of the application of these and other data to the detection of HFCS addition to honey will appear elsewhere (9).

LANDIS W. DONER

JONATHAN W. WHITE, JR.

*Eastern Regional Research Center,
Agricultural Research Service,
Philadelphia, Pennsylvania 19118*

References and Notes

1. N. H. Mermelstein, *Food Technol. (Chicago)* **29** (No. 6), 20 (1975).
2. A. O. Nier and E. A. Gulbransen, *J. Am. Chem. Soc.* **61**, 697 (1939).
3. H. C. Urey, *J. Chem. Soc.* (1947), p. 562.
4. M. M. Bender, *Phytochemistry* **10**, 1239 (1971).
5. B. N. Smith and S. Epstein, *Plant Physiol.* **47**, 380 (1971).
6. The δ¹³C value is defined by

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

The reference standard is Pee Dee belemnite (PDB) carbonate.

7. A. Nissenbaum, A. Lifshitz, Y. Stepek, *Lebensm. Wiss. Technol.* **7**, 152 (1973).
8. C. Hillaire-Marcel, O. Carro-Jost, C. Jacob, in preparation.
9. J. W. White, Jr., and L. W. Doner, in preparation.
10. J. W. White, Jr., M. L. Riethof, M. H. Subers, I. Kushnir, *Composition of American Honeys* (Technical Bulletin 1261, U.S. Department of Agriculture, Washington, D.C., 1962).
11. J. W. White, Jr., in *Honey—A Comprehensive Survey*, E. Crane, Ed. (Heinemann, London, 1975), pp. 157-206.
12. T. Whelan, W. M. Sackett, C. R. Benedict, *Biochem. Biophys. Res. Commun.* **41**, 1205 (1970).
13. We thank J. G. Phillips for statistical counsel.

7 April 1977; revised 20 May 1977