Table 1. The O^{18}/O^{16} and C^{13}/C^{12} maxima, minima, averages, and ranges for *Globigerinoides rubra* and *Globigerina bulloides* at le Castella, Italy. The isotopic values refer to the Chicago Standard PDB-1 (PDB, Pee Dee belemnite).

Species	O^{18}/O^{16} (δ ‰)				C^{13}/C^{12} (δ ‰)			
	Maximum	Minimum	Average	Range	Maximum	Minimum	Average	Range
Globigerinoides rubra	+2.03	−2.03	−0.39	4.06	+1.01	−1.10	+0.21	2.11
Globigerina bulloides	+2.94	−0.15	+1.46	3.09	+0.08	−2.87	−1.23	2.95

G. bulloides, a winter species, indicates a greater influx of fresh water into the Crotone basin during the winter, as fully expected from the meteorological regime of the area. The lighter carbon isotopic composition of both *G. rubra* and *G. bulloides*, in correlation with lighter oxygen isotopic composition, suggests greater runoff during intervals of high temperature, which is in agreement with the known aridity of the Mediterranean during cold ages (*12*).

The greater secular ranges in O^{18}/O^{16} ratios for both *G. rubra* and *G. bulloides* with respect to the C^{13}/C^{12} ratios is a measure of the added temperature effect affecting predominantly the O^{18}/O^{16} ratios. In fact, if secularly varying runoff were the only factor, C^{13}/C^{12} ranges should be equal to or greater than the O^{18}/O^{16} ranges [compare (*11*)].

That the O^{18}/O^{16} minima represent high temperatures is also shown very clearly by the shell morphology of *G. rubra.* In the equatorial Atlantic and the Caribbean, this species is represented by the subspecies *G. rubra rubra* (the classical *G. rubra* of d'Orbigny) during both glacial and interglacial ages (*13*). In the North Atlantic *G. rubra rubra* occurs only during the interglacial ages and is replaced by the more compact *G. rubra gomitulus* during the glacial ages (*13*). In the eastern Mediterranean core 189, *G. rubra rubra* is narrowly restricted to the highest temperature intervals of the interglacial ages, while *G. rubra gomitulus* remains abundant through the rest of the core (*14*). In the section at le Castella under discussion here, *G. rubra rubra* predominates in the intervals having highly negative O^{18}/O^{16} ratios (sample Nos. 4, 16 to 19, 32, 33, 39 to 43, 52, and 61), while *G. rubra gomitulus* predominates in all others. There is strong micropaleontological evidence, therefore, that the O^{18}/O^{16} minima of Fig. 1 represent temperature maxima and not only greater influx of fresh water.

The observed coincidence of O^{18}/O^{16} minima with tripoli-rich layers appears to result from a common cause, namely, higher temperatures and greater fresh water influx. A direct causal relationship between high productivity of diatoms and radiolaria and low O^{18}/O^{16} ratios in the foraminiferal calcite is not supported by the C^{13}/C^{12} ratios, unless one were to believe that productivity is directly proportional to temperature secularly but inversely proportional to it seasonally (lower C^{13}/C^{12} ratios are given by *G. bulloides*, a winter species, than by *G. rubra*, a summer species; see Table 1).

Because of the uncertainty in the oxygen isotopic composition of the Crotone basin water, it is not possible to calculate absolute temperature values from the data of either Table 1 or Fig. 1. Nevertheless, it appears rather well established on both isotopic and micropaleontological grounds that the O^{18}/O^{16} variations represent marked climatic variations occurring while the sediments of the Plio-Pleistocene section at le Castella were being deposited. As previously noticed (*2*), no major temperature change occurs at the Plio-Pleistocene boundary. The amplitude of the O^{18}/O^{16} variations is about 0.7 per mil smaller than that noticed in the late Pleistocene core 189 from the eastern Mediterranean (*14*).

Six major temperature maxima are evident on both isotopic and micropaleontological grounds. If the resulting five major cycles had an average duration similar to that of the temperature cycles of the later Pleistocene [about 50,000 years each (see *15*)], the stratigraphic section of Fig. 1 would represent a time interval of 250,000 years and would have been deposited at an average rate of 10 cm per 1000 years.

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References and Notes

1. M. Gignoux, *Ann. Univ. Lyon* **1**, 28 (1913).
2. C. Emiliani, T. Mayeda, R. Selli, *Bull. Geol. Soc. Amer.* **72**, 679 (1961).
3. C. Roda, *Geol. Romana* **3**, 319 (1964).
4. G. Ruggieri, *Riv. Ital. Paleontol. Stratigr.* **67**, 405 (1961).
5. G. M. Richmond and C. Emiliani, *Science* **156**, 410 (1967).
6. W. D. Bock, *ibid.* **170**, 847 (1970).
7. D. C. Bayliss, *Lethaia* **2**, 133 (1969).
8. L. A. Smith, *Trans. Gulf Coast Ass. Geol. Soc.* **19**, 579 (1969).
9. A. W. H. Bé, *Micropaleontology* **6**, 373 (1960).
10. J. I. Jones, *ibid.* **13**, 489 (1967).
11. S. Epstein, R. Buchsbaum, H. Lowenstam, H. C. Urey, *Bull. Geol. Soc. Amer.* **64**, 1315 (1953); W. G. Mook, thesis, University of Groningen (1968).
12. E. Bonatti, *Nature* **209**, 984 (1966).
13. C. Emiliani, *Micropaleontology* **15**, 265 (1969).
14. ———, *Quaternaria* **2**, 87 (1955).
15. ———, *J. Geol.* **74**, 109 (1966).
16. I am grateful to R. Selli for having made available the samples. Supported by NSF grant GA-15226. Contribution No. 1299 from the School of Marine and Atmospheric Sciences, University of Miami.

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Amorphous Ice: Density and Reflectivity

Abstract. *Amorphous ice prepared under a wide range of conditions has a density, determined from its buoyancy in liquid oxygen, of 0.94 ± 0.02 gram per cubic centimeter, the same as that of ordinary hexagonal ice, with no indication of the glassy superdense ice (2.32 grams per cubic centimeter) reported recently. The diffuse reflectivity shows a small increase as the ice crystallizes at 153°K. This increase is followed by a much larger increase (probably associated with crystal growth) as the sample warms, and the reflectivity reaches a maximum well below the melting temperature. Although the ice deposits appear translucent, the specular reflectivity is low, thus indicating a dull rather than a glassy surface.*

Ice prepared by the condensation of water vapor on a surface cooled to 140°K or below gives only broad bands in its x-ray diffraction and is generally considered to be amorphous (*1*). Recently Delsemme and Wenger (*2*) reported the preparation of "glassy" amorphous ice with a density of 2.32 g/cm³. This very high density was determined by computing the volume of a known weight of ice from photographs of a copper cone with and without the ice deposit. We have visually examined the crystallization of many samples of amorphous ice and have seen no change in volume or other indication of an abnormally high density.

We have now measured directly, from the buoyancy in liquid oxygen or nitrogen (*3*), the density of ten samples of amorphous ice deposited under conditions comparable with those reported for the preparation of "superdense" ice. As a further check on the possibility of a change in density upon crystallization, the diffuse and specular reflectivities were observed during the deposition and subsequent warming of amorphous ice. Any large change in the density of the deposit would be expected to break up the sample and cause a large increase in the diffuse reflectivity.

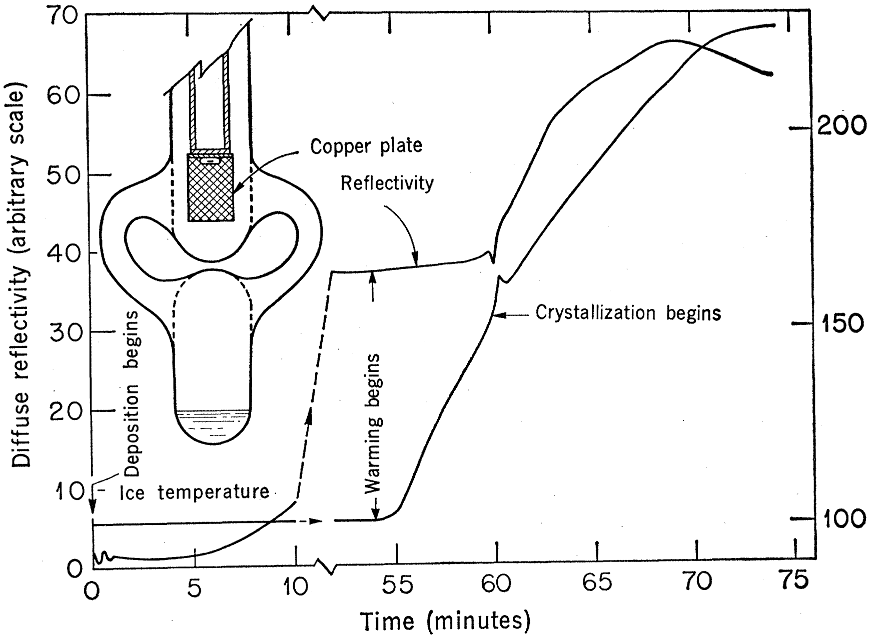

Fig. 1. Diffuse reflectivity of a typical sample of ice during the initial portion of the deposition on a black surface at 101°K, and during subsequent warming. The diagram at left shows the still pot and the copper plate onto which ice is deposited. The plate is fastened to a copper tube which is connected by a housekeeper seal to the inner wall of the Dewar flask above. The glass system is connected by a glass taper to the outer wall of the Dewar flask.

Figure 1 shows the lower portion of the system used for the preparation of amorphous ice. About 1 to 2 ml of pure water (*4*) was placed in the still pot and thoroughly degassed on a high-vacuum system by repeated freeze-pump-thaw cycles. The sample was protected from contamination from the mercury diffusion pump by means of a trap cooled with liquid nitrogen. The upper limit for contamination of the sample by air was estimated to be less than 1 part per million (ppm). After degassing, the Dewar vessel was isolated from the pumping system by closing a stopcock; the still pot was cooled to a temperature in the range −15°C to −30°C, with the exact temperature dependent on the deposition rate desired; and the sample plate was then cooled with either liquid N_2 or liquid O_2. The temperature at which the sample was deposited was measured by means of 0.013-mm Chromel and constantan thermocouple wires cemented near the bottom of the plate with conducting silver-epoxy. Most of the density preparations were made with the plate rotated 90 deg from the position shown in Fig. 1, and with the vapor entry tube nearest to the plate partially blocked. Care was taken to maintain the sample near 77°K while the holding screw was removed and the suspension wire was hooked onto the plate. The density was determined from the following sequence of weighings: (i) the weight of the ice and the plate in liquid oxygen (or nitrogen), (ii) the weight of the ice and the plate in cold nitrogen gas, (iii) the weight of the plate alone in liquid oxygen (or nitrogen), and (iv) the weight of the plate in air. The liquid oxygen was precooled about 1 deg below the boiling point in order to minimize bubble formation on the sample. The liquid oxygen, prepared in the same manner each day by passing oxygen through a coil of tubing immersed in liquid nitrogen, was assumed to contain a small amount of nitrogen, and, therefore, the density of a typical preparation was measured. The density (2.207 g/cm^3) of a short piece of fused quartz tubing was determined from its weight in water at 22°C and in air. Measurement of the buoyancy of the same piece of fused quartz gave 1.140 g/cm^3 for the density of the liquid oxygen used in the ice density measurements. A density of 0.940 g/cm^3 at 89°K was found for each of two samples of ordinary hexagonal ice, one from a laboratory ice-making machine and the other prepared by the slow freezing of degassed pure water. These densities are in good agreement with the values of 1.137 and 0.930 g/cm^3 for oxygen at its boiling point and ice at 84°K, respectively, reported by Dewar (*3*). This same oxygen density is given in a recent review (*5*).

Ten samples of amorphous ice were deposited on aluminum or copper plates at temperatures ranging from 82° to 110°K and deposition rates from 16×10^{-6} to 69×10^{-6} g cm^{-2} sec^{-1} for times of 12 to 120 minutes. The measured densities ranged from 0.89 to 0.98 g/cm^3 and averaged 0.93 g/cm^3. The buoyancy of ice in liquid nitrogen [density (*5*), 0.808 g/cm^3] was used to determine the densities of the first four samples, while the buoyancy in liquid oxygen, considered to be more accurate, was used for the last six samples and gave a density of 0.94 ± 0.02 g/cm^3.

All other samples of amorphous ice, including some prepared in an all-glass system (*6*), floated in liquid oxygen (1.1 g/cm^3) and sank in liquid nitrogen (0.81 g/cm^3).

When samples of amorphous ice were examined visually during warming, the change in appearance from translucent to opaque white was so gradual that it was not possible to determine the time or temperature at which the change occurred. In order to make the observation more quantitative, the copper plate was painted a dull black, light from a tungsten lamp was beamed onto the surface at a 45-deg angle, and light scattered at 90 deg from the surface near the center of the plate was piped to a photomultiplier (RCA 6217). The output of the photomultiplier and the temperature of the plate were recorded continuously. The diffuse reflectivity of a typical sample is shown in Fig. 1. In the first few minutes the deposition rate was very slow and interference colors could be seen to correspond with the oscillations in the reflectivity. Between 5 and 54 minutes after the start of deposition the deposition rate became nearly constant and there was a steady increase in reflectivity. When the thickness of the ice reached ~ 0.3 mm, deposition was stopped and warming was begun. The reflectivity increased very little up to 140°K, where a more rapid increase began; then at ~ 152°K the reflectivity started to decrease. At 153°K the sample temperature rose rapidly, an indication of crystallization, and the reflectivity showed a corresponding rapid but small increase. The reflectivity then continued to increase rapidly and reached a maximum at ~ 212°K. At this point the reflectivity was > 90 percent of that of a surface freshly coated with MgO smoke.

One may speculate that the decrease in reflectivity just before crystallization is due to the glass transition (*7*) where the amorphous solid becomes a viscous liquid. The further increase in reflectivity after crystallization is attributed to crystal growth from dimensions smaller than the wavelength of light to dimensions greater than the wavelength of light.

When the photomultiplier and its light pipe were moved to an angle of 45 deg from the surface to measure specular reflectivity, a thin layer of amorphous ice greatly reduced the light reflected from polished copper, an indication of a dull rather than a glassy surface for the amorphous ice.

Note added in proof: One of the referees of our revised manuscript called our attention to a technical comment by Seiber *et al.* (*8*) on the "Density of low temperature ice" with a reply by Delsemme and Wenger (*9*). Seiber *et al.* determined the density by measuring the thickness of the ice formed by the condensation of a known mass of water on a known surface area. The thickness was determined by interference techniques. They obtained a low value of 0.81 ± 0.02 g/cm^3 as compared with our value of 0.94 ± 0.02 g/cm^3. This difference is reasonable since their method gives the bulk density, an average that includes the density of pores and other voids.

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References and Notes

1. E. Burton and W. Oliver, *Proc. Roy. Soc. Ser. A Math. Phys. Sci.* **153**, 1966 (1935); L. G. Dowell and A. P. Rinfret, *Nature* **188**, 1144 (1960).
2. A. H. Delsemme and A. Wenger, *Science* **167**, 44 (1970).
3. This method was used → J. Dewar [*Proc. Roy. Soc.* (*London*) **70**, 237 (1902)] to measure the density of ordinary ice at the temperature of liquid air.
4. Pure water was prepared by the successive distillation of tap distilled water from acid dichromate, then from alkaline permanganate, and finally in an all-silica still. The water was stored in silica flasks.
5. A. A. Vasserman and V. A. Rabinovich, *Liquid Air and Its Components* (translated from Russian by A. Barouch) (the translation is available from the U.S. Department of Commerce Clearinghouse for Federal Scientific and Technical Information, Springfield, Va., 1970).
6. J. A. Ghormley, *J. Chem. Phys.* **48**, 503 (1968).
7. M. Sugisaki, H. Suga, S. Seki, *Bull. Chem. Soc. Jap.* **41**, 2591 (1968); C. A. Angell and E. J. Sare, *Science* **168**, 280 (1970).
8. B. A. Seiber, B. E. Wood, A. M. Smith, P. R. Müller, *Science* **170**, 652 (1970).
9. A. H. Delsemme and A. Wenger, *ibid.*, p. 654.
10. We are grateful to F. Vaslow for use of the density apparatus, and for his foresight in allowing space for Dewar flasks under the analytical balance. Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.

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Chemically Induced Porphyria: Increased Microsomal Heme Turnover after Treatment with Allylisopropylacetamide

Abstract. *Excessive induction of δ-aminolevulinic acid synthetase in rats after treatment with porphyria-inducing chemicals, such as allylisopropylacetamide, is accompanied by a decrease in microsomal heme and cytochrome P450 concentrations. Measurement of the radioactive decay after labeling of the heme moiety of submicrosomal particles shows increased breakdown of heme in rats treated with allylisopropylacetamide. The effects of allylisopropylacetamide on heme synthesis and heme turnover may be interrelated.*

2-Allyl-2-isopropylacetamide (*1, 2*) and diethyl-1,4-dehydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (DDC) and certain of its analogs (*1, 3*) produce a biochemical situation in rodents mimicking human hepatic porphyria—that is, the hepatic content and excretion of porphyrins and their precursors are increased. This abnormality in both human and chemically induced porphyria is an immediate consequence of the excessive de novo synthesis of hepatic δ-aminolevulinic acid synthetase (ALA-synthetase), the rate-controlling enzyme in porphyrin and heme biosynthesis (*1, 4*).

A larger number of chemicals, characterized by phenobarbital, induce more modest and circumscribed increases in ALA-synthetase in the same animals, seemingly providing heme for increased formation of microsomal cytochrome P450 (*5*). Because of the limited increase of ALA-synthetase, animals so treated do not exhibit the prolonged increases in urinary excretion of δ-aminolevulinic acid, porphobilinogen, and porphyrins which are characteristic of hepatic porphyria. The marked difference in the induction of ALA-synthetase between these drugs and porphyria-inducing chemicals like allylisopropylacetamide and DDC has remained unexplained.

The present studies are based on the following findings. (i) End product control of heme biosynthesis primarily by repression of ALA-synthetase constitutes an important regulatory mechanism in liver (*1, 5*). (ii) Administration of porphyria-inducing chemicals like allylisopropylacetamide and DDC decreases the concentration of heme and of cytochrome P450 in liver microsomes (*6*). We determined whether decreased synthesis or increased degradation is responsible for the decrease in cytochrome P450 and, further, whether this phenomenon is related to the induction of ALA-synthetase.

Male Sprague-Dawley rats of the Holtzman strain (180 to 220 g) were fasted for 24 hours before the beginning and throughout the course of the experiments. After the rats were decapitated, the livers were perfused with isotonic saline in situ; they were then excised, homogenized in 0.25*M* sucrose, and pooled. To assay ALA-synthetase activity in liver homogenates, the incorporation of [1,4-^{14}C]succinic acid (*7*) or [2,3-^{3}H]succinic acid (*8*) into δ-aminolevulinic acid (ALA) was determined and radioactive ALA was isolated by three consecutive chromatographic procedures (*8*). Cytochrome P450 was determined in liver microsomes by the method of Omura and Sato (*9*). δ[3,5-^{3}H]Aminolevulinic acid (588 mc/mmole, New England Nuclear) was injected intravenously in 0.9 percent NaCl in a dose of 10 μc per 100 g of body weight, and the animals were killed at various times after the injection. Microsomes were prepared by the method of Schneider (*10*). The final microsomal pellet was suspended in 0.1*M* KH_2PO_4–K_2HPO_4 (*p*H 7.0), containing 25 percent glycerol (by volume) and 2 m*M* ethylenediaminetetraacetate. The microsomes were then subjected to anaerobic digestion by treatment with subtilopeptidase (E.C. 3.4.4.16; Nagarse, Nagase