Table 2. Pristane in recent marine sediments. Wilkinson Basin, Gosnold Cruise No. 65; 42°24′N, 69°29′W; depth, approx. 255 m. Volden Fjord, Norway, Chain Cruise No. 13, 63°09.5′N, Long. 5°59.8′E. Depth 659 m. All data are based on sediment dried at 110°C. The extract represents the benzene-soluble fraction of the methanol-benzene extraction. Saturated hydrocarbons were eluted from silica gel by normal pentane.

Constituent	Wilkinson Basin	Volden Fjord
$CaCO_3$ (%)	1.49	12.62
C (%)	1.03	2.68
Extract (ppm)	650	1300
Saturated hydrocarbons (ppm)	9.1	44
Pristane (10^{-3} ppm)	13	18

tain no detectable phytane. This suggests that recent oil pollution, which would introduce comparable quantities of pristane and phytane, played no role in the accumulation of the pristane. Rather, this hydrocarbon stems from recent organisms, most likely copepods, directly or through intermediates in the marine food chain. Like the sediments, these organisms contain no phytane.

Thus, based on limited data from two present sediments, phytane and isoprenoid hydrocarbons of lower molecular weight of ancient sediments appear to be postdepositional geochemical products. Phytane probably is formed by conversion of sedimentary phytol, for instance, by clay-catalyzed dehydration to phytadienes, followed by hydrogenation. Apparently, this process is too slow to generate detectable amounts of phytane in the uppermost sediment layer.

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References and Notes

1. C. H. Brieskorn and K. Zimmermann, *Experientia* **21**, 385 (1965); J. D. Mold, *Biochemistry* **3**, 1293 (1964).
2. M. Blumer, M. M. Mullin, D. W. Thomas, *Science* **140**, 974 (1963); *Helgoländ. Wiss. Meeresunters.* **10**, 187 (1964).
3. R. A. Dean and E. V. Whitehead, *Tetrahedron Letters* **1961**, 768 (1961); J. G. Bendoraitis, B. L. Brown, L. S. Hepner, *Anal. Chem.* **34**, 49 (1962).
4. J. J. Cummins and W. E. Robinson, *J. Chem. Eng. Data* **9**, 304 (1964); G Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame, M. Calvin, *Science* **145**, 263 (1965); W. G. Meinschein, E. S. Barghoorn, J. W. Schopf, *ibid.*, p. 262.
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Fast Reactions of Ascorbic Acid and Hydrogen Peroxide in Ice, a Presumptive Early Environment

Abstract. *Nonenzymatic decomposition of hydrogen peroxide proceeded more rapidly in ice than in liquid water. At 5×10^{-7}M ferric chloride or 10^{-8}M cupric chloride, breakdown of hydrogen peroxide was significant at $-11°$ and $-18°$ but negligible at $+1°$C. Ascorbic acid oxidation was faster in ice both with or without added metal ion. Nonparallel effects of metals and* pH *indicate mechanism changes in ice.*

Recent work has shown that several bimolecular reactions proceed more rapidly in ice than in liquid water (*1–6*). Kinetic analyses (*4, 5*) and inhibition by substrate analogs in frozen systems (*6*) suggest that the reaction mechanism has changed on freezing and that the ice structure may participate catalytically. These findings have possible implications in areas ranging from the storage of tissues and food at low temperatures to considerations of the origin of life on earth and elsewhere. We have now extended the studies in frozen solutions to metal ion–catalyzed reactions which behave similarly to enzymatic reactions. These include the breakdown of hydrogen peroxide and the oxidation of ascorbic acid.

For determination of hydrogen peroxide breakdown, an adaptation of the catalase assay method of Bonnichsen, Chance, and Theorell (*7*) was used. Solutions for storage were prepared in deionized distilled water and contained $0.0056M$ H_2O_2, $0.02M$ tris buffer at *p*H 7.2, or $0.02M$ acetate buffer at *p*H 5.5, and the desired amounts of $CuCl_2$ or $FeCl_3$. Freezing was initiated in a dry ice–acetone bath, and the samples were then transferred to $-11°$ or $-18°$C. At half-hour intervals over periods of 3 to 5 hours, samples were thawed rapidly in a bath at room temperature, in the presence of 1-percent sulfuric acid, and titration was performed with $0.006N$ potassium permanganate. The reaction was first-order at all temperatures to at least 50 percent decomposition.

Acceleration of hydrogen peroxide decomposition occurred in ice under all conditions studied. Table 1 shows that at $5 \times 10^{-5}M$ iron the rate in ice ($-11°$) exceeded that in water ($+1°$C) by a factor of 28. Both rates decreased with decreasing iron concentration, although not in parallel. At $5 \times 10^{-4}M$ iron, the rate at $-18°$ was 10 times that at 1°C. At $5 \times 10^{-7}M$ iron, the reaction rate was still significant at $-18°$, but zero at $+1°$C. Lowering the *p*H to 5.5 lowered the rates in the liquid and frozen solutions. The fall at $-18°$, however, was far more abrupt than that at $+1°$C, again indicating dissimilar reaction mechanisms.

Table 2 compares the rates for cupric chloride–catalyzed splitting of hydrogen peroxide. At $-11°$ copper was considerably more effective than iron, although iron catalyzed the reaction somewhat better at 1°C. At $10^{-8}M$ copper, the reaction was appreciable even at $-18°$C ($k = 28 \times 10^{-5}$ min^{-1}). An additional index of the rate enhancement on freezing is the extent of substrate decomposition after 4 days; at $10^{-8}M$ copper, this figure was 78 percent at $-11°$, 50 percent at $-18°$, and only 4 percent at 1°C.

Ascorbic acid oxidation, measured by the spectrophotometric method of Racker (*8*), also followed clean first-order kinetics in frozen solutions. Table 3 compares the rates in liquid and frozen systems containing $10^{-4}M$ ascorbic

Table 1. Rates of Fe(III)-catalyzed splitting of hydrogen peroxide in liquid and frozen systems.

$FeCl_3$ (M)	*p*H	Rate [$10^5 k_{obsvd}$ (min^{-1})]		
		1°C	−11°C	−18°C
5×10^{-5}	5.5	14.9	24.7	17.6
5×10^{-4}	7.2	23.0		227
5×10^{-5}	7.2	9.2	255	
5×10^{-6}	7.2	7.7	96.7	
5×10^{-7}	7.2	0.0	52.2	22.6

Table 2. Rates of Cu(II)-catalyzed splitting of hydrogen peroxide in liquid and frozen systems; *p*H 7.2.

$CuCl_2$ (M)	Rate [$10^5 k_{obsvd}$ (min^{-1})]	
	1°C	−11°C
10^{-5}	0.0	272
10^{-6}	0.0	166
10^{-7}	0.0	130
10^{-8}	0.0	61

Table 3. Rates of ascorbic acid oxidation in liquid and frozen systems. Absorbance read at 245 mμ.

$CuCl_2$ (M)	*p*H	Rate [$10^3 k_{obsvd}$ (min^{-1})]	
		1°C	−11°C
5×10^{-6}	5.5	15.9	56.4
0	5.5	5.0	14.2
5×10^{-6}	5.0	4.6	5.8
0	5.0	3.4	3.8

acid and 0.02M acetate, buffered at pH 5.5 and 5.0. Unlike the hydrogen peroxide decomposition, this reaction proceeded both in the presence and absence of added metal ions. The rate at −11° exceeded that at +1°C under all of these conditions. At pH 7.2, all of the ascorbate oxidations occurred more rapidly than at lower pH levels, the reaction in ice being too rapid to give satisfactory kinetics under the conditions used.

At least two chemical species participate in each of the reactions reported here. The reactants, including molecular oxygen in the case of ascorbic acid oxidation, are clearly brought by freezing into an environment conducive to reaction. Whether this environment is the ice lattice or liquid microinclusions is not clear. However, the kinetic data appear to contradict a mechanism based upon the concentration increases resulting from phase separation.

The types of chemical reactions known to be capable of proceeding at significant rates in ice now include hydrolysis (*1*, *3*), dehydration (*2*), aminolysis (*4–6*), peptide formation (*9*), oxidation, and peroxide decomposition. In view of the facility with which these reactions occur, and as a result of Urey's postulate of a low-temperature origin of the earth (*10*), there is reason to question the assumption that water present only as frost or ice precludes prebiotic chemical evolution. It is evident, for example, that the combination of restricted diffusion and low temperature fails to slow down a number of reactions of biochemical importance. Second, one of the conditions regarded as essential for prebiotic interactions is concentration of various precursor molecules (*11*); the process of freezing could accomplish this concentration. Third, a frozen environment suggests limitations to the assumption that once living organisms have appeared they would consume biochemicals arising *de novo*, thereby perpetuating existing characteristics (L-amino acids, and so forth). In ice, newly formed chemical structures would probably be sequestered from living organisms and might evolve to considerable complexity.

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References and Notes

1. N. H. Grant, D. E. Clark, H. E. Alburn, *J. Am. Chem. Soc.* **83**, 4476 (1961).
2. W. H. Prusoff, *Biochim. Biophys. Acta* **68**, 302 (1963).
3. A. R. Butler and T. C. Bruice, *J. Am. Chem. Soc.* **86**, 313 (1964).
4. T. C. Bruice and A. R. Butler, *ibid.*, p. 4104.
5. H. E. Alburn and N. H. Grant, *ibid.* **87**, 4174 (1965).
6. N. H. Grant and H. E. Alburn, *Biochemistry* **4**, 1913 (1965).
7. R. K. Bonnichsen, B. Chance, H. Theorell, *Acta Chem. Scand.* **1**, 685 (1947).
8. E. Racker, *Biochim. Biophys. Acta* **9**, 577 (1952).
9. N. H. Grant, D. E. Clark, H. E. Alburn, *J. Am. Chem. Soc.* **84**, 876 (1962).
10. H. C. Urey, *Geochim. Cosmochim. Acta* **26**, 1 (1962).
11. J. D. Bernal, in *The Origins of Prebiological Systems and of Their Molecular Matrices*, S. Fox, Ed. (Academic Press, New York, 1965), p. 71.
12. We thank S. Hixson for valuable technical assistance.

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Lactate Dehydrogenase Isozymes: Substrate Inhibition in Various Human Tissues

Abstract. *The total lactate dehydrogenase (LDH) in whole homogenates of various human tissues reacts more similarly toward pyruvate and lactate at 25°C than expected from the marked differences in substrate inhibition at this temperature between isolated, purified LDH-1, and LDH-5. At 37°C, LDH-5 closely resembles LDH-1 in extent of inhibition by substrate. These results are incompatible with the theory that differences in degree of isozyme inhibition by substrate have resulted in predominance of LDH-5 in anaerobic tissues and predominance of LDH-1 in aerobic tissues.*

Two principal theories have been proposed to elucidate the functional significance of LDH isozymes. Based on inhibition at 25°C of isolated LDH-1, purified by concentrations of pyruvate to which LDH-5 is resistant (*1*), the first theory states that LDH-5 predominates in anaerobically metabolizing tissues because, in high pyruvate concentrations, it functions more efficiently than does LDH-1 (*2*). By contrast, LDH-1 is claimed as the predominant isozyme in aerobic tissues where high substrate concentrations do not develop (*2*). The second theory, consistent with the first but more general, maintains that individual isozymes fulfill distinctive roles by virtue of being situated in various regions of a cell. Subcellular localization of isozymes would depend on their different kinetic properties, as well as on the metabolic organization of the particles with which they associate (*3*). Recently various isozymic forms have been demonstrated to be subject to feedback control by compounds in divergent metabolic pathways (*4*).

The purpose of this report is to describe the observation that the total LDH activity in whole homogenates of several human tissues behaves even at the unphysiologic temperature of 25°C in a qualitatively and quantitatively similar fashion toward substrate. These tissues do not reveal the marked differences in substrate inhibition expected and predicted by the first theory in view of the dissimilarity at 25°C between isolated, purified LDH-1 and LDH-5. These kinetic studies performed on whole homogenates have obvious limitations from which investigations on isolated, purified isozymes do not suffer. However, it is shown that isolated, purified LDH-1 and LDH-5 at the physiologic temperature of 37°C exhibit similar kinetic behavior on exposure to increasing concentrations of substrate.

Human heart, psoas muscle, liver, and pancreas, obtained within 12 hours of death, were homogenized in a Waring blendor at 4°C (one part of tissue to five parts of phosphate buffer pH 7.4, 0.4M). The homogenates were centrifuged at 15,000g for 1 hour; the supernatant was assayed spectrophotometrically at 340 mμ for LDH activity, both by oxidation and reduction of the coenzyme at 25°C. Between 2 and 5 μl of homogenate provided sufficient activity when assayed with varying concentrations of pyruvate (4×10^{-5} to $4 \times 10^{-2}M$) and lactate (1×10^{-4} to $3.6 \times 10^{-1}M$). The volume of the assay mixture was kept constant at 3 ml by appropriate additions of phosphate buffer (pH 7.4, 0.4M).

The substrate solutions were adjusted to pH 7.4 before addition to the cuvettes. Nicotineamideadenine di-