

the crystalline hydrocarbon hartite. The pattern of *Abies amabilis* is like that obtained for abietic acid which had been heated 24 hours at 90°C. Resins from some of the *Pinus* species gave distinctly different patterns. These include *Pinus monophylla*, one of the varieties of *Pinus caribaea*, and one of the varieties of *Pinus oocarpa*. Among the resins from angiosperm trees, the pattern for *Styrax benzoin* is essentially that of cinnamic acid.

It may be mentioned here that Pelletier and Walter (3) isolated a white crystalline hydrocarbon by dry distillation of succinite (Baltic amber) and that this hydrocarbon was believed to be identical with the naturally occurring hydrocarbon idrialite. Strunz and Contag believe idrialite is identical with picine (C₂₄H₁₄). When more such identifications and correlations are made they should aid in revealing genetic relationships between fossil and modern resin-producing trees.

Metastable Superheated Ice in Liquid-Water Inclusions under High Negative Pressure

Abstract. *In some microscopic inclusions (consisting of aqueous liquid and vapor) in minerals, freezing eliminates the vapor phase because of greater volume occupied by the resulting ice. When vapor fails to nucleate again on partial melting, the resulting negative pressure (hydrostatic tension) inside the inclusions permits the existence of ice I crystals under reversible, metastable equilibrium, at temperatures as high as +6.5°C and negative pressures possibly exceeding 1000 bars.*

Liquids can be metastably stretched, under certain conditions, to occupy a larger volume (at a lower density) than that called for at equilibrium. The pressure on the liquid is then less than the (hypothetical) vapor pressure and often less than zero, yielding negative pressures (*I*) that will persist as long as nuclei for a vapor phase are absent. Such negative pressures are of considerable significance in various biological systems (2) and are particularly important in boiling or cavitation. I have found no reference to the sustained metastable existence of ice I above its normal melting point of 0°C, such as I have observed in frozen fluid inclusions (3). These inclusions yielded possibly the highest negative pressures ever recorded in a static liquid system, although they are still well below the theoretical tensile strength of water (4).

Microscopic aqueous fluid inclusions in minerals can be frozen to determine the salinity of the inclusion fluid

Correlation is needed between results obtained with x-ray diffraction and those of botanists and organic chemists using infra-red absorption spectroscopy and other techniques. It is also necessary to crystallize and identify more individual compounds from these resins for use as standards in the x-ray diffraction study.

JUDITH W. FRONDEL
Hoffman Laboratory, Harvard University, Cambridge, Massachusetts

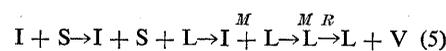
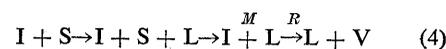
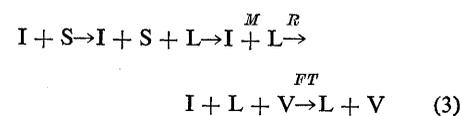
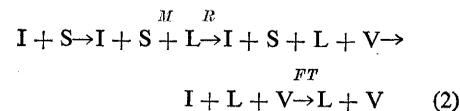
References and Notes

1. The specimens were obtained from collections of the Harvard University Mineralogical and Botanical Museums, the mineralogical collection of the U.S. National Museum, the mineralogical collection of the British Museum, from Dr. J. Langenheim, and from field collecting at the Arnold Arboretum in Jamaica Plain, Mass.
2. H. Strunz and B. Contag, *Neues Jahrb. Mineral. Monatsh.* 1965-1, 19 (1965).
3. Pelletier and P. Walter, *J. Prakt. Chem.* 1, 114 (1844).
4. This investigation was made possible by a scholarship from the Radcliffe Institute, Cambridge, Mass.

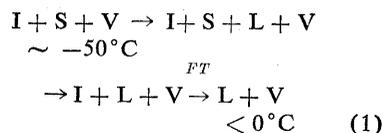
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freeze with only about two-thirds as much expansion (7).

When frozen saline inclusions are warmed, there should be a net reduction in volume as the ice and salt mixture melts and dissolves to form ice and brine. (The thermal expansion of the liquid is generally much smaller, as the temperatures are near that of maximum density.) At equilibrium via sequence 1 this volume decrease corresponds to the growth of a bubble consisting of water vapor and gases formerly present as solids such as CO₂·5¾H₂O (8). Fluid inclusions are very small systems, exceptionally free from nuclei (9); hence they exhibit several metastable phenomena. Once the vapor phase has been eliminated, renucleation is sluggish. Depending upon the quantities of the various phases and the temperatures attained before nucleation, four metastable sequences are possible, and each has been found in natural inclusions:



from its freezing temperature, that is, from the depression of the freezing point (5). The equilibrium sequence of phase changes, usually obtained when a frozen inclusion (consisting of saline solution and vapor bubble at room temperature) is warmed, is



where I represents ice crystals, S represents salt crystals (mainly NaCl·2H₂O), L is liquid, V is vapor, and FT is freezing temperature.

The volume expansion on formation of ice eliminates the vapor bubble in those inclusions, formed at relatively low temperature, which contain only a small vapor bubble (6). Inclusions of pure water, in which the vapor is up to 8 percent (by volume) at room temperature, are filled with ice on freezing; the strong brines in some inclusions

where *M* indicates a metastable assemblage under negative pressure, and *R* indicates a rapid phase change (10).

Sequence 2 is difficult to recognize with certainty, due to the mass of solids present, but the moment of nucleation in 3, 4, and 5 is readily recognized by an apparently instantaneous appearance of a vapor bubble in the liquid and an almost instantaneous melting of ice (Figs. 1 and 2). Because the metastable assemblages may persist for as long as an hour, it is difficult to observe the actual melting of ice when the negative pressure is released. On several occasions, however, other observers and I have witnessed it, and I am certain that there is a definite interval of time (estimated at 0.1 to 0.2 second) over which the melting occurs after nucleation. The melting front must advance through the ice crystal at rates on the order of 200 μ per second.

Sequences 2 and 3 permit valid determinations of the freezing temperature after nucleation of vapor, but 4 and

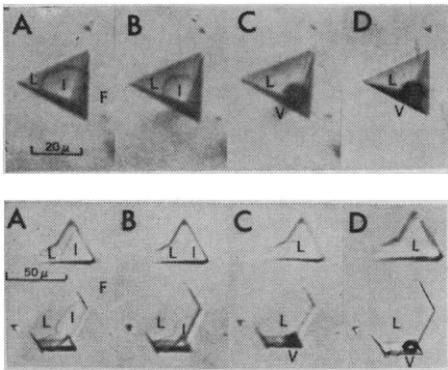


Fig. 1 (top). Superheated ice in fluid inclusions in fluorite sample 4, in sequence from left to right, equilibrated at: A, + 1.00°C; B, + 3.55°C; C, + 3.75°C; D, + 22°C. Time between individual photographs was 5 to 15 minutes. I, ice; L, liquid water solution; V, vapor bubble; F, fluorite host crystal. Inclusion 3a, via sequence 4, with sudden nucleation of vapor bubble between B and C, at + 3.65° ± 0.1°C. Fig. 2 (bottom), Superheated ice in fluid inclusions in fluorite sample 4, equilibrated as in Fig. 1, at: A, + 1.35°C; B, + 2.95°C; C, + 3.45°C; D, + 22°C. Inclusion 5 (lower row), via sequence 4, with sudden nucleation between B and C, at + 3.15° ± 0.25°C; inclusion 5a (upper row), via sequence 5, with last ice melting between B and C at + 3.15° ± 0.25°C, with no nucleation of vapor bubble.

5 do not. Even though the ice in sequences 2 and 3 before nucleation is at subzero temperatures, it is still "superheated," in that it is in metastable equilibrium with a brine at a temperature too high for the salinity (11). The presence of superheated ice is most obvious in inclusions of low salinity that follow sequence 4 or 5 and have true freezing temperatures near 0°C. In these, metastable ice crystals persist at temperatures as high as + 6.2°C via sequence 4, and + 6.5°C via sequence 5. Most such inclusions contain an estimated 90 percent by volume of ice at 0°C, and many show as much as 70 percent at + 2°C and 50 percent at + 3°C.

Metastable ice persists for more than 4 hours in some inclusions. Because the sample plates are 1 to 2 mm thick, and are completely immersed in rapidly flowing, thermostated acetone, thermal equilibration is complete within seconds. In view of the calibration procedures, the stated temperatures are probably accurate to < 0.1°C (9).

Nucleation of vapor appears to be somewhat random in time and temperature because of considerable in-

consistency on reruns of a given inclusion. However, as expected from the phase volume relationships involved, those inclusions which yield metastable assemblages on one run are more likely to show them on another run. Also, the maximum temperature of existence of metastable ice via sequences 4 and 5 is seldom 10°C above the stable freezing temperature, and usually is only a few degrees higher. The presence of nucleation sites with specific properties is suspected in some inclusions that show a tendency to nucleate in a relatively narrow temperature range. Although inherent variation among inclusions invalidates direct comparisons, for any given co-genetic group of inclusions the probability of nucleation increases greatly with increase in temperature.

Several hundred inclusions or groups of inclusions in many samples of minerals from a variety of geological occurrences exhibit metastable ice (Table 1). Similar metastability is found in inclusions in barite, quartz, calcite, and sphalerite from these and numerous other localities. Repeat runs with the same sequence give essentially identical temperatures for phase changes involving reversible equilibria (stable or metastable); but because the temperatures of the irreversible, rapid phase changes by means of sequences 2, 3, 4, or 5 are controlled by nucleation, repeat runs with the same sequence (Table 1) generally give differing temperatures (12). Nucleation of vapor in some inclusions by sequence 5 does not occur until these inclusions are held at room temperature for hours or days (13). The assemblages before nucleation of vapor are in reversible (though metastable) equilibrium, because the ice crystals can be made to grow or melt (but not nucleate) on appropriate temperature changes.

Ice was identified by the following criteria (5): (i) an index of refraction

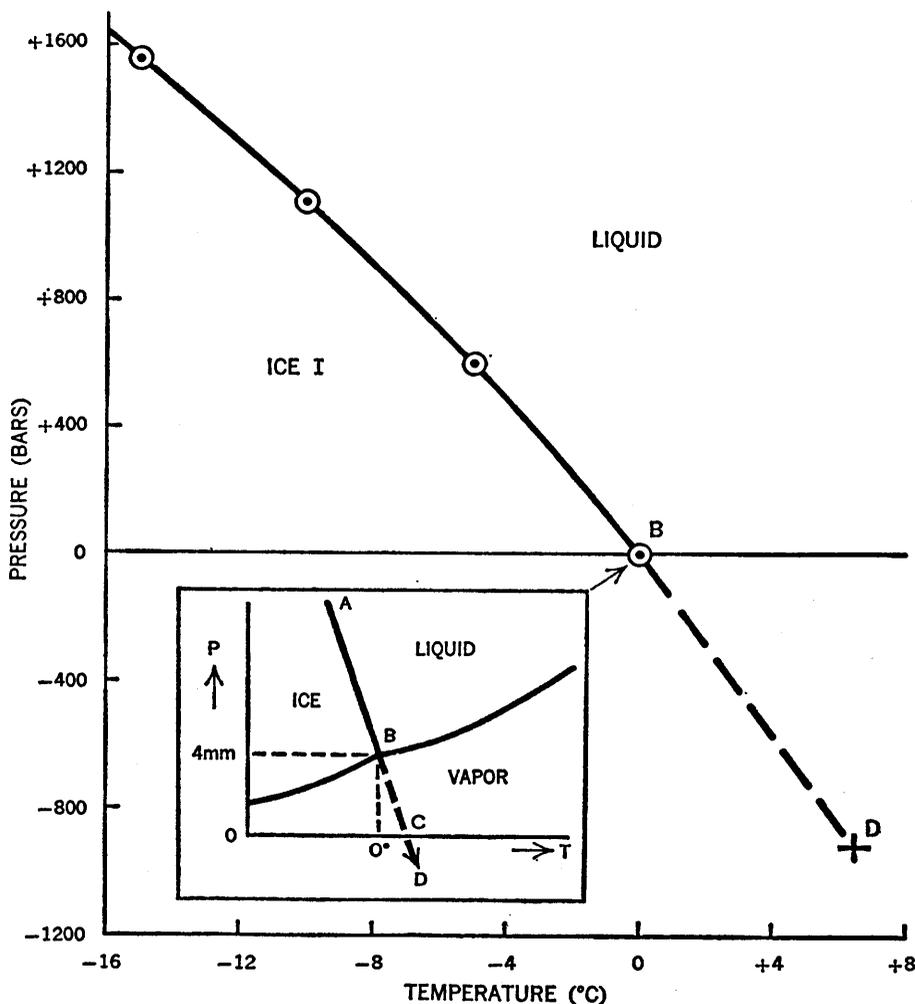


Fig. 3. Melting curve for ice I [circles from Bridgeman (14)], with a possible (straight-line) extrapolation into the region of negative pressure to the maximum observed temperature of superheated ice (D). The insert (diagrammatic) shows the stable phase boundaries (solid lines) in the immediate vicinity of the triple point B: low-salinity inclusions containing only ice and water follow the metastable extension of line AB (in the pure system water); along BC these inclusions show slightly superheated ice at positive pressures lower than that of the triple point B; beyond C they enter into the region of negative pressure.

appreciably less than that of the solution into which it melts; (ii) very low birefringence, in the correct range for ice; (iii) characteristic growth of two parallel plates from opposite sides of rounded grains on temperature drop; (iv) volume decrease during melting; (v) the general crystallization temperature range; and (vi) the gross fluid composition, by analysis of numerous other samples. Only rarely do the optical properties of the host mineral permit determination of optical sign and orientation relative to crystal habit; the few data thus obtained also agreed with the properties of ice I. The quantity of ice in any given inclusion is so small (10^{-8} to 10^{-9} g) that the thermal effects of the sudden phase change would be difficult to recognize. Also, the temperature drop from melting is counteracted by the heat release when the tension is released.

The magnitude of the negative pressure within inclusions having superheated ice is estimated to be at least 900 bars, and possibly over 1000 bars, but there are numerous uncertainties. A straight-line extrapolation (Fig. 3), derived from the Clausius-Clapeyron equation and data on water and ice at 0°C (14), yields a value of over 900 bars at $+6.5^{\circ}\text{C}$, and any curvature is likely to increase this value. The validity of this extrapolation is uncertain, because it requires the assumptions that the heat of melting and the thermal expansions and extensibilities of ice and water under negative pressure are comparable to the equivalent values under positive pressure. Although these assumptions are at least approximately true (15), there is considerable evidence that "premelting" and "prefreezing" effects are common and are particularly expected in ice and water (16). Because the inclusions are not pure water but contain salts in solution, the "univariant" curve for them would be displaced to the left on Fig. 3; this results in even greater negative pressures for a given temperature above 0°C .

Estimates based on measurements of the degree of filling of inclusions showing behavior according to sequence 5, with the assumption that extensibility of liquid water under negative pressure is equivalent to its compressibility under positive pressure, yield comparable negative-pressure values. An additional assumption in this method of estimation, that the surrounding container is rigid, is obviously invalid but of minor effect.

Previous experimental studies provide much lower estimates of the maximum sustained tensile strength of liquids (17). When water is cooled in glass Berthelot tubes, contraction develops negative pressure as high as 200 bars, although later examination of the method has shown that these data require several corrections (1, 2, 18).

Some fern cells develop negative pressures as high as 400 bars (2). By centrifuging liquids in capillary tubes, Briggs has brought water to a maximum of 277 bars negative pressure at $+6^{\circ}\text{C}$ (19), and various cavitation procedures yield negative pressures of the order of 100 bars (20). Rapid superheating of small quantities of fluid

Table 1. Fluorite samples whose inclusions exhibit negative pressure. Temperatures represent the midpoints between pairs of measurements establishing the phase change, with a difference as indicated by the stated uncertainty. Arrows indicate change in percent ice by volume at nucleation. Sample 1: East Prospect mine, Central Kentucky fluorite district; colorless, coating scale-nohedral calcite; ER 62-24. Sample 2: Cuesta molybdenum mine, New Mexico; multicolored; ER 62-41. Sample 3: Barite fluorite vein, Rampart Range, Colorado; multicolored; ER 62-49a. Sample 4: Poncha Springs, Colorado; pale green; ER 62-50. Sample 5: Quatras Palmas, Coahuila, Mexico; banded purple; ER 62-52. Samples 6 through 9: Treak Cliff, Castleton, Derbyshire, England; banded "blue john" crystals; strongly saline inclusions in interior of crystals, low-salinity inclusions in outermost rim; ER 62-132a, b, c, and d.

Inclusion No.	Stable freezing temperature ($^{\circ}\text{C}$)	Rapid phase change from metastable equilibrium ($^{\circ}\text{C}$)		Melting of last ice crystal under metastable equilibrium ($^{\circ}\text{C}$)
		Via sequence 3	Via sequence 4	Via sequence 5
<i>Sample 1</i>				
15d	~ -16			-4.1 ± 0.3
12	-3.71 ± 0.02	-4.9 ± 0.1	-3.3 ± 0.2	
<i>Sample 2</i>				
3e	-0.15 ± 0.06			-0.05 ± 0.05
3h	-1.00 ± 0.10			$+1.65 \pm 0.2$
3k	-0.15 ± 0.05		$+4.5 \pm 0.1$	
1f	< -28.0			-10.8 ± 0.3
1c	-17.9 ± 0.2			-9.55 ± 0.05
<i>Sample 3</i>				
3	~ -0.3		$+0.32 \pm 0.12$	
6	~ -0.3		$+2.35 \pm 0.30$	
16	~ -0.3		$+5.60 \pm 0.35$	
13	~ -0.3		$+6.23 \pm 0.18$	
21	~ -0.3			$+5.78 \pm 0.13$
<i>Sample 4</i>				
3a	-0.1 ± 0.05		$+3.65 \pm 0.1$	
5	-0.1 ± 0.05		$+4.17 \pm 0.5$	
	-0.1 ± 0.05		$+4.55 \pm 0.3$	
	-0.1 ± 0.05		$+3.15 \pm 0.3$	
5a	-0.1 ± 0.05			$+3.15 \pm 0.3$
25	-0.1 ± 0.05	-0.90 ± 0.3		
7	-0.1 ± 0.05			$+6.32 \pm 0.12$
				$+6.43 \pm 0.04$
				$+6.50 \pm 0.15$
12a	-0.1 ± 0.05		$+6.10 \pm 0.13$	
1	-0.1 ± 0.05		$+4.41 \pm 0.04$	
1	-0.1 ± 0.05		$+1.33 \pm 0.23$	
<i>Sample 5</i>				
20b	-1.31 ± 0.01	~ -1.35	-0.95 ± 0.05	
20b	-1.31 ± 0.01		-0.60 ± 0.05	
21f	-3.75 ± 0.05		-4.05 ± 0.05	
21e	-2.90 ± 0.07		-2.0 ± 0.2	
<i>Sample 6</i>				
25	-1.00 ± 0.03	-1.32 ± 0.03		
17	-1.06 ± 0.04	-1.78 ± 0.28		
16e	~ -1.5		-0.80 ± 0.05	
<i>Sample 7</i>				
6	-0.97 ± 0.03	-2.2 to -3.9	-0.52 ± 0.03	~ -0.2
6a	~ -16	~ -20	~ -8.8	~ -5
<i>Sample 8</i>				
10	-17.7 ± 0.1			-11.8 ± 0.3
1b, d, f	-1.22 ± 0.03	~ -2.0		
<i>Sample 9</i>				
4d	-16.95 ± 0.05	-19.6 ± 0.8		

Inclusion 12: by sequence 3, $60 \rightarrow 15\%$ ice. Inclusion 13: by sequence 4, this and similar inclusions had approximately 5% ice at $+6^{\circ}\text{C}$. Inclusion 5: triplicate runs. Inclusion 7: duplicate runs. Inclusion 12a: 25% ice at $+5.65^{\circ}\text{C}$. Inclusion 1: duplicate runs. Inclusion 20b: duplicate runs; via sequence 3, $50 \rightarrow 2\%$ ice; heated at 0.05° per minute from -2.3° . Inclusion 17: by sequence 3, $60 \rightarrow 40\%$ ice. Inclusion 16e: by sequence 4, $70 \rightarrow 0\%$ ice. Inclusion 6: group of cogenetic primary inclusions. Inclusion 6a: plane of cogenetic secondary inclusions. Inclusion 4d: by sequence 3, $50 \rightarrow 15\%$ ice.

may yield negative pressures of about 50 bars, although ions in solution may greatly lower the temperature for nucleation (21). Negative pressures up to 250 bars have been obtained by supersaturation of liquids with respect to a gas (22).

The theoretical limits for the tensile strength of water vary, according to the method of calculation, from < 1000 to > 15,000 atmospheres (4). The great difference between the theoretical and experimental values can be attributed mainly to experimental difficulties in eliminating gas nuclei (23) that can be deactivated by high pressures, strong centrifuging, and shock waves from impact, as well as by care to avoid all hydrophobic substances (24). Inclusions showing negative pressures have probably been subjected to moderate to high (positive) internal pressures upon the original freezing, and negative pressures develop even though the freezing of water may itself form gas nuclei (25).

A negative melting curve is required for a substance to exhibit metastable superheating under negative pressures. Several curious phenomena in the system water may be explained by the fact that such negative pressures raise the melting point. The well-known Tyndall stars that form within ice were recognized as regions of low or zero pressure (26), and transient superheating of the ice to approximately +0.3°C just prior to their formation has been reported (27). Fabian observed a significant decrease in the rate of melting of ice placed under uniaxial tension (28).

Clean liquid water may be greatly supercooled except when subjected to cavitation. Hickling (29) suggested that nuclei of high pressure ice VI or VII were formed, at transient temperatures as high as 160°C, due to extremely high positive pressures upon bubble collapse. Wylie proposed, however, that extremely high negative pressures just before cavitation locally raised the melting point of ice I well above 0°C, thereby yielding ice nuclei (30).

Measurements of single crystals of ice show uniaxial tensile strengths two orders of magnitude lower (31) than the negative pressures in these inclusions. An ice crystal in water under negative pressure is in a very different environment from that of a normal specimen undergoing tensile strength determination. Not only is the tension perfectly uniform triaxially, but the

(anisotropic) crystal is free to deform in any direction without restraint and without influencing the tensile stress at its surface. Although the much lower measured tensile strength may be due mainly to the presence of crystal imperfections in the large samples used, the possibility might be considered that nucleation of the vapor bubble may be due to failure of ice in tension. This is not the case, however, as the ice crystal was never seen to rupture, and the gas bubble usually nucleated apart from the ice crystal.

It is important to know whether nucleation of vapor occurs at specific sites on the inclusion wall or in the fluid itself and whether the maximum tension varies appreciably with the nature of the host mineral (or daughter minerals) and hence with the interfacial energy. Also, ordering of water molecules for some distance from the inclusion wall could yield a relationship to inclusion size (32). Adequate data on these problems have not been obtained.

EDWIN ROEDDER

U.S. Geological Survey,
Washington, D.C. 20242

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- The temperature above which vapor should be present at equilibrium can be determined by recooling, after vapor nucleation, to the point where expansion from growth of ice just eliminates the vapor bubble.
- In two inclusions in a sample of quartz (courtesy of Professor G. Dessau) from Macchia Casella Manciano, Tuscany, Italy, nucleation of vapor and elimination of ice during two very slow runs (equilibration for 30 minutes after each 0.5°C rise from +1° to +4° or +5°C) occurred at a temperature consistently higher, by about 1°C than in eight runs in which the temperature was raised uniformly at 5 minutes per degree.
- Failure to nucleate the vapor phase in water-filled inclusions under mild negative pressure (due to cooling from the original homogeneous condition at trapping) may allow metastability to persist for geological times in samples formed at temperatures under 100°C.
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33. I thank Allen V. Heyl, D. R. Shawe (U.S. Geological Survey), and T. D. Ford (University of Leicester) for the mineral samples (Table 1); G. Czamanske, W. O. Smith, S. W. Benson, C. Mongan, and L. Trefethen for advice; and especially John P. Creel, who first noticed ice crystals at abnormal temperatures, prepared the polished plates, and made most of the temperature measurements. Publication authorized by the director, U.S. Geological Survey.

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Chromosomal Damage in Human Leukocytes Induced by Lysergic Acid Diethylamide

Abstract. *Addition of lysergic acid diethylamide to cultured human leukocytes resulted in a marked increase of chromosomal abnormalities. The distribution of chromosome breaks deviated significantly from random, with an accumulation of aberrations in chromosome No. 1. Cytogenetic investigation of a patient extensively treated with this drug over a 4-year period for paranoid schizophrenia showed a similar increase in chromosomal damage.*

The induction of chromosomal abnormalities by various exogenous agents has been studied extensively (1, 2). In addition, compounds with specific pharmacologic and chemotherapeutic value cause chromosome damage (3). The psychotomimetic agent lysergic acid diethylamide (LSD-25), when added to cultures of human peripheral leukocytes, produces a marked increase in the frequencies of chromosomal breaks and rearrangements compared to untreated cultures.

Chromosomal preparations were made from cultures of whole blood with a microtechnique and standard procedures (4). All cultures were incubated for 72 hours at 37°C, and colcemide (0.05 µg/ml) was added for the last 2 hours of culture to arrest cells at metaphase. Lysergic acid diethylamide was dissolved in sterile distilled water and added to the cultures in various concentrations (100, 50, 10, 1, 0.1, 0.01, and 0.001 µg/ml of culture) for different periods of exposure before harvest (48, 24, and 4 hours). Concentrations of 100 and 50 µg/ml caused cellular degeneration and suppressed mitosis so

that the number of analyzable cells was insufficient. Leukocytes obtained from two healthy individuals (one male and one female) were treated with LSD-25 at final concentrations of 10, 1, 0.1, 0.01, 0.001 µg/ml for 48, 24, and 4 hours. Each concentration and exposure time was repeated twice. The controls consisted of untreated cultures from these two individuals as well as from four additional persons, two males and two females.

Several slides from each culture were prepared and coded by individuals who did not participate in the microscopic scoring of the cells. It was hoped that 25 metaphases per slide could be obtained to yield a total of 200 cells for each concentration and time period. However, in some of the treated cultures, we could not find this number of cells. Well-spread mitoses were selected under low magnification (× 250), and chromosomes were scored under oil-immersion phase-contrast microscopy (approximately × 1560). Once a cell was selected under low power, it was included in the study.

Abnormalities were scored as breaks only if a clear discontinuity of the chromatid was visible. Breaks were classified as "chromatid" if only one chromatid was affected and "isochromatid" if both sister chromatids were broken at the same location. Both of these types of abnormalities were scored as single breaks. Single fragments were included with chromatid breaks while "double" fragments were scored as isochromatid breaks. Dicentric chromosomes and "translocation" configurations were considered as containing two breaks. Attenuated, pale-staining chromosomal regions, other than the normal secondary constrictions, were scored separately as "gaps" but were not included in the calculation of breakage rates. Whenever possible, each break was assigned to a given identifiable chromosome or chromosome group ac-

ording to the Denver classification (5).

Since there was no observable difference in the responses of the two individuals, the data for each treatment were pooled. Table 1 illustrates the distribution of chromosomal abnormalities observed for various exposure times and concentrations of the drug. At least a twofold increase in the rate of chromosomal breaks over the control rate was evident for all treatments (except 0.001 µg/ml for 4 hours). A relationship between dose and response existed; however, this appeared to be time dependent. The highest concentration (10 µg/ml) caused greater damage in shorter incubation times, an indication that the longer exposure may have caused cellular destruction. The same effect is also noted with a concentration of 1.0 µg/ml in the 48-hour treatment. Conversely, with 0.001 µg/ml, more chromosomal damage was evident at longer exposure times, while, with the 4-hour exposure with this dosage, a direct reduction in the number of chromosome breaks was observed.

Table 2 depicts the distribution of chromosome breaks among the various identifiable chromosomes or chromosome groups. The test of significance indicates a nonrandom distribution of breaks ($P < 0.001$), with a disproportionate accumulation of anomalies in chromosome No. 1. The array of expected values is based on random breakage per unit of chromatin as calculated from the Denver measurements (5). Studies of other agents inducing nonrandom breakage of human chromosomes have demonstrated "hot spots" in the heterochromatic regions of chromosome No. 1 [for example, the centromere and secondary constriction (2)]. Lysergic acid diethylamide also shows an apparent affinity for these chromosomal regions.

At most concentrations, the greatest damage was induced by 24- and 48-hour exposure periods. Although the

Table 1. Distribution of chromosomal breaks induced in cultured human leukocytes by various dosages and times of exposure to LSD-25. Data are given as breaks per number of cells. Figures in parentheses denote breaks per cell.

Time before harvest (hours)	Dosage (µg/ml)				
	10	1	0.1	0.01	0.001
48	15/164 (0.091)	13/194 (0.067)	41/125 (0.328)	19/200 (0.095)	27/195 (0.138)
24	22/200 (0.110)	46/125 (0.368)	34/175 (0.194)	28/175 (0.160)	22/175 (0.216)
4	38/150 (0.253)	18/200 (0.090)	23/200 (0.115)	28/200 (0.140)	10/200 (0.050)
Control	34/925 = (0.037)				