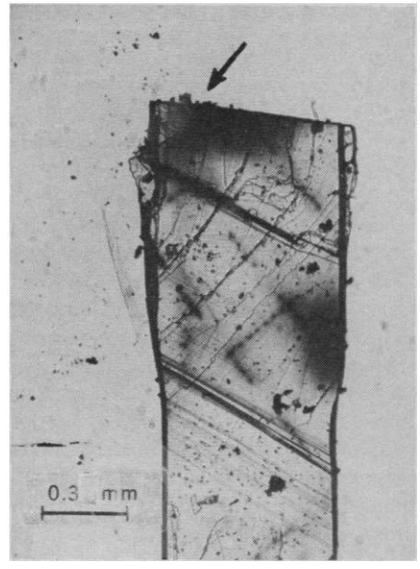
Fig. 2. Optical micrograph showing blue coloration in the area of Fig. 1a (arrow) after electron microscopy.



superlattice and CaF_2 matrix reflections, as noted in the schematic diffraction net of Fig. 1d. On looking at the superlattice pattern, one can see that the defect symmetries indicated by the arrows in Fig. 1a are coincident with the ⟨220⟩ directions of the superlattice.

Because of the very small size of the sample fragments examined, little identifiable color was observed. Nearly all samples appeared completely colorless. However, after examination in the electron microscope, distinct and intense blue-colored regions were observed in the samples. Figure 2 shows the region of the sample from which the images and the selected-area electron diffraction pattern of Fig. 1 were obtained, illustrating the associated coloration. It is apparent that 200-kv electron-beam irradiation of the fluorite has induced additional defects and defect agglomeration, and that the defect agglomeration is directly responsible for the residual coloration. This seems to be a clear implication of color centers in natural fluorite, analogous to the results reported by Seitz on alkali halides (*4*).

The CaF_2 structure can be visualized as a cubic array of F^- ions with Ca^{2+} ions at every other body-centered position. Fluorite is therefore intrinsically a calcium-vacancy-defect structure. However, Frenkel defects (or F^- vacancies) in the anion sublattice are likely to be favored and preferentially formed by electron bombardment because the anions, though physically larger than the cations, have a small coordination number. In addition, since the dielectric properties of fluorite give rise to a charge-storage capacity, numerous electrons from the 200-kv beam are available for trapping by the anion vacancies to form color centers. These centers can easily migrate and agglomerate as a result of the thermal activation of the electron beam and the open structure of the CaF_2 lattice.

Although this study appears to report the first direct observations of the ordering of vacancy (color-center) aggregates in a mineral, I should mention that vacancy ordering has also been observed in nonstoichiometric NiAl which was found to be a defect CsCl (body-centered cubic) structure with the vacancies forming triangular arrangements in the (111) plane (*6*). The investigation reported here shows that electron microscope observations of an ordered defect aggregate superlattice array, presumably color-center aggregates, are directly associated with the observed coloration in fluorite. These results appear to be the first direct observations of color-center aggregates and the first direct evidence of color-center activity in a colored mineral system. These results will be presented in greater detail elsewhere (*7*).

L. E. MURR

Department of Metallurgical and Materials Engineering, New Mexico Institute of Mining and Technology, Socorro 87801

References and Notes

1. K. C. Dunham *et al.*, Eds., *Fluorspar* (Department of Scientific and Industrial Research, Memoirs of the Geological Survey, His Majesty's Stationery Office, London, ed. 4, 1952), vol. 4; K. Przibram, *Irradiation Colours and Luminescence* (Pergamon, London, 1956); E. Roedder, A. V. Heyl, J. P. Creel, *Econ. Geol.* **63**, 336 (1968); E. Roedder, *ibid.* **66**, 98 (1971).
2. S. R. Titley and P. E. Damon, *J. Geophys. Res.* **67**, 4491 (1962).
3. F. Seitz, *Rev. Mod. Phys.* **18**, 384 (1946).
4. ———, *ibid.* **26**, 7 (1954).
5. S. Amelinckx, *Direct Observation of Dislocations* (Academic Press, New York, 1964); L. E. Murr, *Electron Optical Applications in Materials Science* (McGraw-Hill, New York, 1970).
6. P. Delavignette, H. Richel, S. Amelinckx, *Phys. Status Solidi* **13**, 545 (1972).
7. L. E. Murr, in preparation.
8. Supported in part by the U.S. Atomic Energy Commission through a research contract from Sandia Laboratories, Albuquerque, New Mexico. I thank L. Taylor for granting me permission to visit the Bingham fluorite site.

16 July 1973; revised 5 October 1973

Ethane Evolution: A New Index of Lipid Peroxidation

Abstract. *Homogenates of mouse liver and brain at 37°C spontaneously formed lipid peroxides and simultaneously evolved ethane. α-Tocopherol, a lipid antioxidant, blocked ethane formation. When mice were injected with carbon tetrachloride (a liquid prooxidant for liver), the animals produced ethane. Ethane evolution in vivo was stimulated by prior administration of phenobarbital and it was diminished by prior injection of α-tocopherol. These data suggest that ethane production may be a useful index of lipid peroxidation in tissue homogenates and in intact animals.*

There is widespread belief that lipid peroxidation may be a factor in certain forms of tissue pathology, particularly under the extreme conditions of experimental vitamin E deficiency (*1*). Recknagel and co-workers have presented evidence for peroxidation of microsomal lipids during CCl_4 poisoning in experimental animals (*2*) and in humans (*3*). We now report that the spontaneous formation of lipid peroxides in homogenates of mouse tissue shaken in air at 37°C is accompanied by evolution of ethane, a hydrocarbon gas. In in vivo studies, administration of CCl_4 to mice provoked ethane evolution. Since α-tocopherol (vitamin E, a lipid antioxidant) blocked spontaneous formation of ethane in vitro and diminished CCl_4-induced ethane formation in vivo, it appeared that lipid peroxidation was linked to the formation of the hydrocarbon gas.

Homogenates of mouse liver and brain were prepared in nine volumes of ice-cold isotonic saline buffered with phosphate at *p*H 7.4 (*4*). Samples (10 ml) were incubated in air with shaking at 37°C in 25-ml Erlenmeyer flasks that had been sealed with rubber caps (*5*). Samples (0.5 or 1.0 ml) of air atmosphere were removed with a gas-tight plastic syringe, equipped with a side-venting needle (*6*). These samples were analyzed immediately by gas chromatography (*7*). Lipid peroxidation was evaluated with the 2-thiobarbituric acid reaction, which measures malonaldehyde evolved as a breakdown product of peroxidized lipids (*8*).

For in vivo studies, five mice were

sealed into a 2.4-liter vacuum desiccator in an air environment. The animals were placed on nickel-chrome wire gauze beneath which was a layer of Baralyme granules (*9*) which functioned to remove respiratory CO_2. Removal of CO_2 created a partial vacuum, which drew fresh O_2 into the chamber from an outside supply. Access to the desiccator was through a three-way stopcock mounted on the desiccator outlet. To remove a sample of chamber atmosphere, the stopcock was turned to close off the O_2 supply, and the chamber contents were mixed by drawing back and forth ten times with a 50-ml syringe mounted on the stopcock; then a small plastic syringe was substituted and 1.0 ml of chamber air was removed for gas chromatographic analysis.

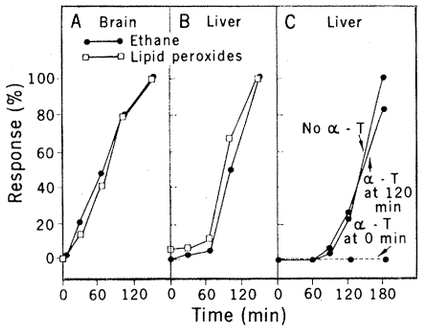
In the in vitro system, there was a lag period of variable length before the evolution of ethane was detected in liver homogenates; a similar lag period was not evident in brain homogenates. In all instances, however, the appearance of ethane was accompanied by lipid peroxidation (malonaldehyde formation) and the time courses for the accumulation of the two were closely correlated. In the representative example shown in Fig. 1A, lipid peroxidation and ethane evolution both proceeded essentially linearly from zero time in brain, while, as shown in Fig. 1B, there was a lag period of approximately 1 hour in liver. The addition of α-tocopherol to either system at zero time (Fig. 1C) completely prevented ethane formation and simultaneously blocked lipid peroxidation. However, when the same amount of α-tocopherol was added at 2 hours, at which time lipid peroxides were present, there was no major effect on the subsequent formation of ethane (Fig. 1C). The latter experiment linked the action of α-tocopherol to the process of lipid peroxidation. The experiment excluded the possibility that α-tocopherol acted at some step subsequent to lipid peroxidation.

The evolution of ethane by animals treated with CCl_4 was studied (Fig. 2). Control animals did not produce ethane but animals treated with CCl_4 did. Some animals were treated beforehand with either phenobarbital or α-tocopherol (see legend to Fig. 2). Ethane evolution was potentiated by phenobarbital and suppressed by α-tocopherol ($P < .01$ at 90, 120, and 150 minutes; Student's t-test).

There is evidence that CCl_4 is metabolized by the hepatic microsomal enzyme system to free radicals, perhaps $CCl_3\cdot$ and $Cl\cdot$, which initiate the lipid peroxidation reactions [(*2, 10*); compare (*1*)]. Phenobarbital potentiates the hepatotoxicity of CCl_4, presumably by inducing the CCl_4-metabolizing enzymes of the smooth endoplasmic reticulum (*11*). In contrast, α-tocopherol is a free radical trapping agent which prevents formation of lipid peroxides (*1*). In experimental animals, α-tocopherol protects against the hepatotoxicity of CCl_4 (*12*). In our in vivo experiments (Fig. 2), the relative rates of ethane evolution by mice treated with CCl_4 appeared to be directly correlated with conditions that governed the degree of susceptibility of liver to lipid peroxidation (namely, increased with phenobarbital and decreased with α-tocopherol).

Another hydrocarbon gas, ethylene, is evolved by intact plant cells and is a putative hormone in plants (*13*). There is evidence for the participation of oxidized unsaturated lipid in this process (*14*). A potential relationship between lipid peroxidation and the ubiquitous presence of ethylene in the plant kingdom led us to investigate whether ethylene would be produced during peroxidation of mammalian cells (*15*). We found that mammalian tissues, in contrast to plant tissues, evolved mainly ethane, although small amounts of ethylene were also detected (ethylene production was about 10 percent of ethane production or less).

Previously, Horvatt *et al.* (*16*) observed the formation of saturated hydrocarbon gases, including ethane, during the oxidation of methyl linoleate in a purely chemical system. Lieberman and Hochstein (*17*) reported that peroxidized rat liver microsomes gave rise to ethylene, but only when ascorbate and cupric ions were added. Ram Chandra and Spencer reported spontaneous formation of trace quantities of ethylene from subcellular particles from rat liver and intestinal mucosa (*18*) and evidence for ethylene in human breath (*19*). Additionally, there is a report that ethylene is exhaled by tumor-bearing animals (*20*). Beauchamp and Fridovich (*21*) described the production of ethylene from methional mediated by xanthine oxidase and



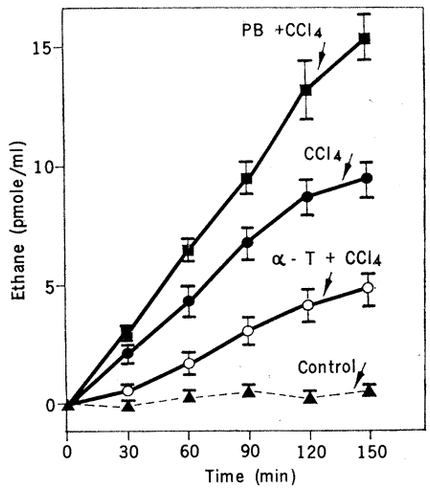


Fig. 1 (left). Lipid peroxidation (malonaldehyde formation) and ethane evolution in homogenates of brain or liver from Paris R III mice. Data are expressed as percentages of the maximum concentrations observed in each experiment. The malonaldehyde concentrations at 2.5 hours in experiments (A) and (B) were 23.8 and 14.6 nmole, respectively, per milliliter of homogenate (*8*). The final ethane concentrations, in nanomoles per milliliter of gas phase, were (A) 0.14 at 2.5 hours, (B) 0.15 at 2.5 hours, and (C) 0.30 at 3.0 hours. α-Tocopherol (*23*) was added to a concentration of 1 μg/ml in experiment (C). When α-tocopherol was added at zero time, lipid peroxides did not accumulate. The gas volume in each flask was 19 ml. Abbreviation: α-*T*, α-tocopherol. Fig. 2 (right). Ethane (per milliliter of chamber air) evolved in vivo by groups of five male Swiss-Webster mice (average weight, 25 g). The gas volume in the chamber was 2.4 liters. Animals were injected intraperitoneally with 0.5 ml of CCl_4 (3.2 g/kg) in light mineral oil (Squibb), or with the vehicle alone (controls). The animals had been treated in advance (three intraperitoneal injections at 42, 24, and 18 hours earlier) with α-tocopherol (25 mg/kg) in 0.9 percent NaCl (weight to volume) solution containing 1 percent (by volume) Tween 80 (*23*), or with the saline–Tween 80 vehicle only. Additionally, some animals received sodium phenobarbital (80 mg/kg) in 0.9 percent saline or vehicle only (four intraperitoneal injections at 66, 42, 24, and 18 hours earlier). The data are the means and standard errors for the experimental ($N = 7$) and control groups ($N = 4$). When animals were not first treated with the saline–Tween 80 vehicle, data similar to those shown in the figure were obtained in the control and CCl_4 groups. Abbreviations: *PB*, phenobarbital; α-*T*, α-tocopherol.

free radicals; previously, Lieberman *et al.* (*22*) described the generation of ethylene from methional or methionine mediated by ascorbate plus cupric ion in a model system.

Our studies have shown that ethane production is a characteristic of spontaneously peroxidizing mouse tissue in vitro and that ethane formation from mice in vivo can be provoked by CCl_4. The amounts of ethane evolved in vivo were correlated with conditions that either increased (phenobarbital pretreatment) or decreased (α-tocopherol pretreatment) the susceptibility of liver to lipid peroxidation. These data suggest that the study of evolved ethane may provide a means to detect and, perhaps, to monitor ongoing pathological lipid peroxidation changes in vivo.

CAROLINE A. RIELY*
GERALD COHEN
College of Physicians and Surgeons, Columbia University, New York 10032
MORRIS LIEBERMAN
U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland 20705

References and Notes

1. B. Century, M. H. Horwitt, P. Baily, *Arch. Gen. Psychiatry* **1**, 420 (1959); H. Zalkin and A. L. Tappel, *Arch. Biochem. Biophys.* **88**, 113 (1960); A. L. Tappel, *Ann. N.Y. Acad. Sci.* **203**, 12 (1972). There is no unanimity of opinion concerning the biologic antioxidant role of vitamin E nor the mechanism of CCl_4-induced hepatotoxicity; for an opposing point of view see J. Green, *ibid.*, p. 29.
2. R. O. Recknagel and A. K. Ghoshal, *Lab. Invest.* **15**, 132 (1966); R. O. Recknagel, *Pharmacol. Rev.* **19**, 145 (1967).
3. S. Hashimoto, E. A. Glende, Jr., R. O. Recknagel, *N. Engl. J. Med.* **279**, 1082 (1968).
4. J. V. Dacie, *The Hemolytic Anemias, Congenital and Acquired* (Churchill, London, 1954), p. 476.
5. We used Vacutainer stoppers (Becton-Dickinson, Rutherford, N.J.).
6. The use of a needle with a side vent (22 gauge, Becton-Dickinson) prevented clogging of the orifice on repeated penetration through rubber.
7. Two gas chromatographs were used, a Beckman GC-5 equipped with a 12-foot (3.6-m) alumina column and a Hewlett-Packard 5750 equipped with a 6-foot (1.8-m) Poropak N column, both with flame ionization detectors. Ethane was observed both in vitro and in vivo with both instruments. Retention times for methane, ethane, and ethylene were 1.0, 1.7, and 2.1 minutes, respectively (alumina, 130°C); 0.5, 1.4, and 1.2 minutes, respectively (Poropak, 90°C); and 0.3, 0.7, and 0.7 minutes, respectively (Poropak, 130°C). For the in vivo experiments presented in Fig. 2 (Poropak N), analyses were carried out at the highest instrumental sensitivity setting, which yielded a 50 percent deflection of the recorder for an ethane concentration of 15 pmole/ml. An unknown substance present in room air and traveling with the same retention time as ethane was distinguished from ethane by passing gas samples through a 15-cm length of Tygon tubing packed with molecular sieve (8/12 mesh, Supelco, Bellefonte, Pa.). This procedure trapped the ethane but passed the unknown substance, which was present in amounts that were constant in any experiment (as determined by zero time, final, and selected midpoint readings), but varied from day to day in the range of 3 to 6 pmole of ethane equivalents per milliliter.
8. A portion of a trichloroacetic acid extract was heated with 2-thiobarbituric acid and the absorbancy was measured at 532 nm [J. Bunyan, J. Green, E. E. Edwin, A. T. Diplock, *Biochem. J.* **77**, 47 (1960)]. This procedure measures malonaldehyde either initially present or evolved under the conditions of the test [F. Bernheim, M. C. Bernheim, K. M. Wilbur, *J. Biol. Chem.* **174**, 257 (1948); L. K. Dahle, E. G. Hill, R. T. Holman, *Arch. Biochem.* **98**, 253 (1962); W. G. Niehaus and B. Samuelsson, *Eur. J. Biochem.* **6**, 126 (1968)]. We used as a primary standard 1,1,3,3-tetraethoxypropane (Eastman Kodak, Rochester, N.Y.) which is hydrolyzed to malonaldehyde under the test conditions.
9. Baralyme is barium hydroxide lime USP with mimoza Z and ethyl violet indicators from National Cylinder Gas Division, Chemetron Corp., Chicago, Ill.
10. T. C. Butler, *J. Pharmacol. Exp. Ther.* **134**, 311 (1961); T. F. Slater, *Nature (Lond.)* **209**, 36 (1966); H. Remmer, *Am. J. Med.* **49**, 617 (1970); T. F. Slater and B. C. Sawyer, *Biochem. J.* **123**, 805 (1971); *ibid.*, p. 815; *ibid.*, p. 823.
11. R. C. Garner and A. E. M. McLean, *Biochem. Pharmacol.* **18**, 645 (1969); R. J. Stenger, R. N. Miller, J. N. Williamson, *Exp. Mol. Pathol.* **13**, 242 (1970); P. Chopra, S. Roy, V. Ramalingaswami, N. C. Nayak, *Lab. Invest.* **26**, 716 (1972).
12. E. L. Hove, *Arch. Biochem.* **17**, 467 (1948); C. H. Gallagher, *Nature (Lond.)* **192**, 881 (1961); N. R. DiLuzio and F. Costales, *Exp. Mol. Pathol.* **4**, 141 (1965).
13. S. P. Burg, *Annu. Rev. Plant Physiol.* **13**, 265 (1962); L. W. Mapson, *Biol. Rev.* **44**, 155 (1969).
14. M. L. Lieberman and L. W. Mapson, *Nature (Lond.)* **204**, 343 (1964).
15. G. Cohen, C. A. Riely, M. Lieberman, *Fed. Proc.* **27**, 648 (1968).
16. R. J. Horvat, W. G. Lane, H. Ng, A. D. Shepherd, *Nature (Lond.)* **203**, 523 (1964).
17. M. Lieberman and P. Hochstein, *Science* **152**, 213 (1966).
18. G. Ram Chandra and M. Spencer, *Nature (Lond.)* **197**, 366 (1963).
19. ———, *Biochim. Biophys. Acta* **69**, 423 (1963).
20. M. T. Kakanov, *Vopr. Med. Khim.* **6**, 158 (1960); *Chem. Abstr.* **56**, 1900 (1962).
21. C. Beauchamp and I. Fridovich, *J. Biol. Chem.* **245**, 4641 (1970).
22. M. Lieberman, A. T. Kunishi, L. W. Mapson, D. A. Wardale, *Biochem. J.* **97**, 449 (1965).
23. *dl*-α-Tocopherol (Calbiochem, San Diego, Calif.) was prepared as a mixture in four parts Tween 80 by volume (Mann Research Labs, New York). Stock dilutions were prepared as required in 0.9 percent NaCl (weight to volume).
24. A preliminary report of part of these studies has been presented (*15*). We thank D. MacNamee and J. Colvil for expert technical assistance. Supported in part by PHS grants HE-01045 and NS-05184 and by the Cardiac Monitoring Fund, Medical Service, Presbyterian Hospital, New York. Reprint requests to G.C.
* Present address: Yale–New Haven Medical Center, New Haven, Conn. 06510.

24 August 1973

Industrial Air Pollution: Possible Effect on Lung Cancer

Abstract. *Higher lung cancer mortality rates occurred in males living in certain heavily industrialized areas of Los Angeles County, California. These areas were characterized by elevated concentrations of benzo*[a]*pyrene and other polynuclear aromatic hydrocarbons of primarily industrial origin in the soil and air.*

Because of the increasingly large number of deaths due to lung cancer, there has been continued interest in the etiology of this disease. During the 1960's several epidemiologic studies (*1*) showed a strong relation between lung cancer and cigarette smoking. The effects of other forms of air pollution on the incidence of lung cancer were also investigated. Occupational studies incriminated specific airborne pollutants (*2*). Urban and rural studies implicated air pollution in general (*3*, *4*). Hammond and Selikoff (*5*) suggested that a synergistic interaction of smoking and industrial, neighborhood, and community air pollution was instrumental in causing lung cancer.

Interest in the possible effect of neighborhood air pollution on lung cancer mortality led us to investigate the geographic distribution of this disease in Los Angeles County. Before the analysis of the mortality data the 26 health districts within the county were grouped into 13 study areas with reasonably homogeneous air pollution profiles, as defined by the Los Angeles Air Pollution Control District (*6*). Populations at risk for these 13 areas were

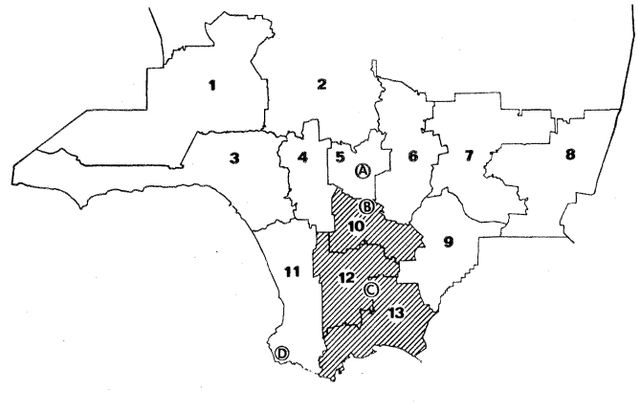


Fig. 1. Map of Los Angeles County showing (shaded area) the three contiguous areas where the lung cancer mortality rate for 1968 and 1969 exceeded 70 per 100,00. The numbers indicate study areas (this report), the letters pollution sampling stations (*12*).