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

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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.
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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.

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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

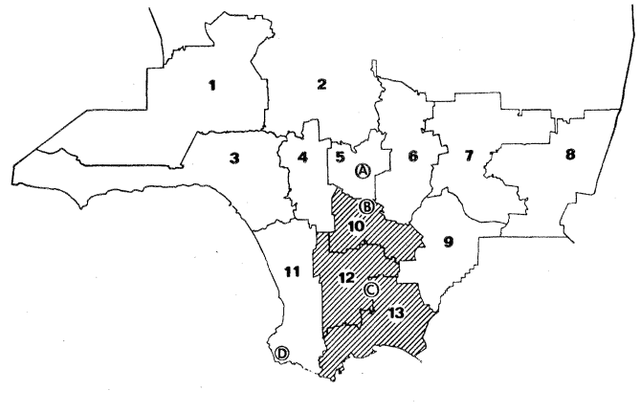

Fig. 1. Map of Los Angeles County showing (shaded area) the three contiguous areas where the lung cancer mortality rate for 1968 and 1969 exceeded 70 per 100,00. The numbers indicate study areas (this report), the letters pollution sampling stations (*12*).

determined from the 1970 census data (*7*). Caucasians comprised the majority of the Los Angeles County population and were widely dispersed through all 13 study areas. To minimize racial differences the analysis was performed on Caucasian deaths only. The percentage of Mexican-Americans, included in the Caucasian subpopulations, varied by 10 to 35 percent between study areas. The age-adjusted lung cancer mortality rates for Caucasian males and females were calculated for the 13 areas from the Los Angeles County death certificates (*8*). The age-adjusted rates were standardized to the U.S. 1970 census population (*9*) by the direct method. A socioeconomic class index from 1 to 5, calculated from the average income and education level, was assigned to each census tract by using a modification of the two-factor Hollingshead index (*10*). Socioeconomic classes 1 to 5 contained 9, 27, 27, 32, and 5 percent, respectively, of the Los Angeles County population of male whites.

The age-adjusted lung cancer rates in 1968 and 1969 for male Caucasians in the 13 study areas ranged from 43 to 75 per 100,000 (Table 1). In areas 10, 12, and 13 the mortality rate was 70 per 100,000 or greater. These three areas are contiguous, are located in south-central Los Angeles County (see Fig. 1), and are populated predominantly by those in the middle and lower socioeconomic classes. The lung cancer mortality rates for Los Angeles County were 1.73 times higher in the lower than the upper socioeconomic classes. However, the excess of lung cancer still remained in the three geographic study areas when all areas were compared for only socioeconomic classes 3 to 5 (Table 1), or for socioeconomic class 4 alone. The percentages of Mexican-Americans living in areas 10, 12, and 13 were close to the Los Angeles County mean. Thus, lower lung cancer rates in Latin males, as reported elsewhere (*11*), could not have explained the excess of lung cancer in these study areas. Analyses of the 13 study areas for age-adjusted mortality rates of all other cancers combined and for all causes of death showed no similar clustering. Age-adjusted mortality rates for esophageal, stomach, colon, pancreatic, bladder, and prostatic cancers, leukemia, and Hodgkin's disease also failed to show clustering. Lung cancer mortality rates for female Caucasians did not show any clustering.

The Caucasian male population of areas 10, 12, and 13 was 499,609. There were 667 lung cancers in these males in the 2-year period under study. If the populations of these three areas had experienced age-specific rates similar to those in the other ten areas in Los Angeles County, approximately 476 lung cancers, or 95 fewer per year, would have been observed. Thus, there was an apparent 40 percent excess of male lung cancers in these three study areas for 1968 and 1969.

Table 1. Lung cancer mortalities (N) and age-adjusted lung cancer mortality rates (R/100,000) (1968 to 1969) by geographic area for male Caucasians in Los Angeles County.

Area	All socioeconomic classes		Socioeconomic classes 3, 4, and 5	
	N	R/100,000	N	R/100,000
1	259	48	129	51
2	289	50	201	57
3	252	49	144	54
4	216	49	196	50
5	281	63	269	63
6	225	43	168	45
7	177	56	138	65
8	97	52	53	51
9	192	53	148	55
10	210	70	191	71
11	231	57	149	64
12	173	75	162	72
13	284	70	257	73
All	2886	55	2205	59

The elevated lung cancer rates in the south-central area of Los Angeles County seem to correlate with results of air and soil sampling measurements taken during 1971 and 1972 by Gordon and Bryan (*12*). They found that concentrations of polynuclear aromatic hydrocarbons in the air and soil differed widely between four sampling stations (A, B, C, and D) in different parts of the county (Fig. 1). The highest concentration of benzo[*a*]pyrene in air and soil was found at station C, situated in the center of the three study areas showing increased lung cancer mortality. The benzo[*a*]pyrene concentration of 3.5 ng/m^3 at station C was approximately five times greater than would have been expected from automotive exhausts alone (*12*). Gordon and Bryan (*12*) suggested that the benzo[*a*]pyrene excess was the result of effluents from the petroleum and chemical industries concentrated in the area. This possible relation between lung cancer and atmospheric benzo[*a*]-pyrene is consistent with earlier findings from England (*3*). Any effect of neighborhood air pollution on lung cancer mortality would probably have to reflect the result of many years of exposure. While the air sampling measurements in 1971 and 1972 from these areas may have been representative of the previous decades, measurements from earlier periods were not available.

It is possible that more smoking or occupational exposure may be related to the excess of lung cancer. Since smoking and occupational history data were not available from the mortality records, the effect of these two factors could not be directly analyzed. Cigarette smoking is inversely related to social class, those in the lower classes smoking more (*13*). The excess of lung cancer in the same geographic areas persists when only the data for the lower socioeconomic classes are analyzed; this reduces the possibility that the excess can be fully explained by differences in smoking (*13*). The possibility of geographic differences in smoking was further weakened by the absence of a similar geographic pattern for cancers of the larynx, bladder, esophagus, or pancreas—cancers also related to smoking (*1*).

It seems unlikely that occupational exposure alone could account for the excess lung cancer deaths. A preliminary review of death certificates for members of the union serving the petrochemical industry did not reveal an excess of lung cancer. We are unaware of any other occupation unique to this area that could be associated with this excess mortality.

The most likely explanation would be a synergistic action between smoking and neighborhood air pollution. The absence of a geographical variation in female lung cancer would be compatible with this hypothesis and similar synergism has been suggested before for smoking and air pollution (*3*, *14*).

HERMAN R. MENCK
JOHN T. CASAGRANDE
BRIAN E. HENDERSON
Department of Pathology, University of Southern California School of Medicine, Los Angeles 90033

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DNA Synthesis and Mitosis in Well-Differentiated Mammalian Cardiocytes

Abstract. *Incorporation of [^{3}H]thymidine into nuclei of heart cells of 2-day-old rats indicates that neonatal cardiac cells containing well-aligned myofibrils synthesize DNA. In these highly differentiated cells, neither the presence of contractile proteins nor their organization into myofibrils inhibits either DNA synthesis or mitosis.*

The relation between DNA synthesis and mitosis and the manufacture of cell-specific proteins has been the subject of numerous studies. In many tissues, differentiation occurs only in cells that no longer divide (for example, nerve cells, keratinizing cells, and reproductive cells). The suggestion that production of myofibrils in striated muscle may inhibit cell division is based on evidence obtained in studies of skeletal muscle (*1*, *2*). Skeletal muscle cells containing myofibrils do not divide (*3*).

Unlike skeletal muscle, embryonic cardiac cells synthesize DNA and divide after myofibrils have appeared (*4–8*). With autoradiography, DNA synthesis has been demonstrated in differentiating cardiac cells of chick embryos (*4*, *5*). Mitotic figures have been observed in electron micrographs of chick heart cells containing a few myofibrils (*6*, *7*). Chacko (*9*) has observed mitosis in electron micrographs of rat cardiocytes during late gestation and at birth. Therefore, in differentiating embryonic cardiac muscle, the appearance of contractile proteins inhibits neither DNA synthesis (*5*) nor mitosis (*6–8*).

Chacko (*9*) has stated that rat myocardial cells do not undergo mitosis once the myofilaments become organized into well-defined myofibrils. However, postnatal cardiac cells of the rat contain well-defined myofibrils (*10*). In a recent review, Zak (*11*) cites evidence indicating that heart muscle cells divide up through 3 weeks of postnatal life. Claycomb (*12*) has recently reported that DNA biosynthesis in cardiac muscle of the rat ceases by the 17th day of postnatal development, which correlates temporally with an almost total loss of cytoplasmic DNA polymerase activity.

Can neonatal cardiac cells that are more highly differentiated than embryonic heart cells synthesize DNA and divide? To examine this question, we studied the hearts of 2-day-old

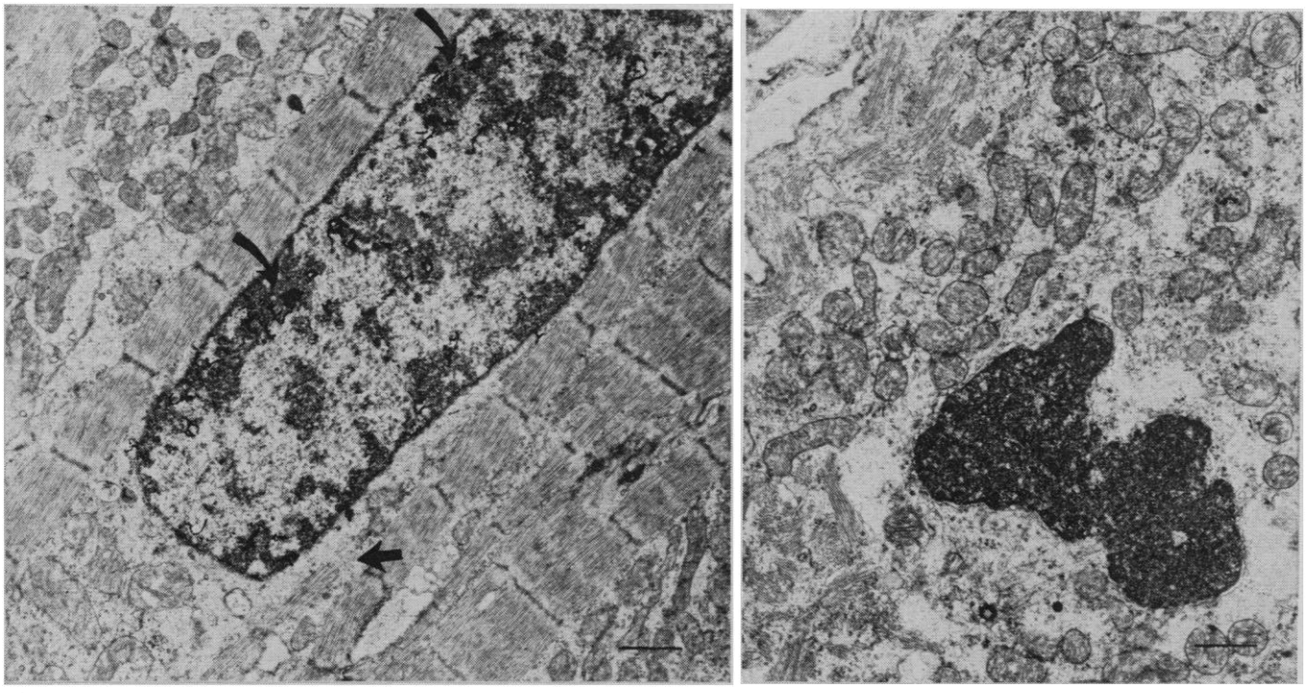

Fig. 1 (left). Typical ventricular myocardial cell of neonatal rat containing mitochondria, granular endoplasmic reticulum, polyribosomes, microtubules, Golgi vesicles, sarcoplasmic reticulum, and numerous myofibrils aligned in the long axis of the cell. The large nucleus contains developed silver grains (curved arrows), indicating incorporation of [^{3}H]thymidine. In one myofibril, the Z band (arrowhead) can barely be seen, although adjacent I and A bands of this myofibril can be seen. Fig. 2 (right). Ventricular cell in telophase. Chromosomes have moved toward the centriole and the nuclear envelope is reforming. Bundles of thick and thin filaments are grouped at random in this mitotic cell. The Z bands are not seen, and the organization of myofilaments into myofibrils is lost. Scale in each figure, 1 μm.