MEDICAL INVESTIGATION OF AIR POLLUTION

Laboratory Methods

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THE LABORATORY investigation of the effects of air pollutants on health may be compared with studies of the effects of micro-organisms on health. The agents concerned with both are apparently limitless. The many and varied atmospheric pollutants, their ubiquity, their ever-changing status in a physically and chemically dynamic atmosphere, and the variations in human response unite to make a study of their biological effects extremely difficult. Clinically, this is so even during transient disaster periods when pollutant concentrations are high, the emission sources are limited, and epidemiological surveys can be undertaken. Studies initiated when pollutant levels are below the threshold for the production of immediate clinical symptoms and at a time when epidemiologic efforts have proved to be of limited value would appear to be well nigh impossible of success. Experimental investigations appear equally dismal. Since the problem of atmospheric pollution varies in accordance with geographical, meteorological, and local industrial conditions, the pollutants under investigation must necessarily be limited to those representing a specific situation. Any program methodology, however, should be basic and with slight modifications capable of application to most studies. This report outlines an attempt to develop a research plan for the study of morbid effects attributable to a local air-pollution situation in Los Angeles County, California.

The fundamental requirement for a study of this type is an adequate background of chemical and engineering knowledge which will permit duplication of atmospheric pollutants in a readily available and easily controllable laboratory tool form. Only within the recent past have studies in this direction been partially successful. On both the clinical and the experimental level, physiologic and pathologic studies fall far short of satisfaction because of the nonspecific and quite subtle

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changes that may occur in response to experimental pollutant exposure at realistic concentrations.

In establishing the physical, chemical, and engineering criteria for the synthetic reproduction of smog, an awareness of pollutant sources alone may be insufficient, since a photochemically active atmosphere can result in the production of many new products. These may be quite unlike pollutants at their sources.[1] This dynamism is of equal significance to medical investigation and to the basic chemical and engineering analytic facets of the problem, as it appears that many of the morbid effects produced in the exposed population are attributable to the new products formed in the atmosphere rather than to the pollutants as they are emitted into the air. The physical science and biological aspects of the problem are discussed separately for convenience only. The actual pursuit of the research requires, of course, simultaneous investigation of both.

EXPERIMENTAL METHODS

Chemical.—In our studies the basic biological aim has been directed at duplicating in experimental animal species the experience that the population undergoes when exposed to smog. Since the human exposure is limited primarily to the exposed mucous membranes, skin, and upper respiratory tract, it is apparent that the artificial reproduction of naturally occurring air pollutants, both qualitatively and quantitatively, is necessary in a readily available laboratory tool form.

Inhalation chambers in which temperature, humidity, and pollutant concentration can be controlled have been used in the studies on animal species. Air pollutants have been studied singly, in combination, and as they occur in whole smog. The artificial smog used was prepared according to a modification of the method of Dr. Shepherd, of the National Bureau of Standards.[2] Since our synthetically polluted atmosphere was a dynamic one, in a manner similar to the naturally occurring one, it became apparent that prior to any biological studies a series of repeated analytical determinations would be necessary so as to observe the entire life cycle of smog from the pollutant source through its build-up and finally to its destruction. This cycle, of course, is a naturally occurring one, and smog composition varies on the basis of the time of day, the geographical location of sample collection, and varying meteorological conditions.

A chief characteristic of the Los Angeles smog is its marked oxidizing capacity.[3] This property is attributable to many groups of compounds in the air. These include ozone, ozonides, organic peroxides, oxides of nitrogen, and aldehydes. This characteristic can be measured, and the results, expressed as total oxidants, provide an index to atmospheric pollution. While this is adequate for a quantitative determination of the intensity of atmospheric pollution, it is far too limited to be of use in a biological investigation. Not only do the individual oxidants vary in their potency, but some in addition to their oxidation effects may carry a toxicity intrinsic in their chemical structure. Further, many of the oxidants act either synergistically or in a potentiating manner upon one another. Consequently, it is important that, consistent with technical knowledge, not only must the total oxidants in the natural and synthetic atmospheric environments be measured but also the percentage contribution of each of the oxidizing substances must be determined. For this a series of reagents have been used in the manner of a screening test, as shown in the following tabulation.

Reagent		Oxidant
HCL	Remove epoxides & reactive alkaline material	....
HI	....	Ozone Ozonides Peroxides
KI	....	Ozone Ozonides
NaOH	Remove acid reacting materials, including organic acids	....
$NaHSO_3$	....	Aldehydes
Reduced phenolphthalein	....	Fast-reacting total oxidants
Alpha naphthylamine Sulfanilic acid	....	Oxides of nitrogen
Explosimeter	....	Hydrocarbons

In initiating our background research plan, a series of questions were formulated. 1. What is the chemical nature of the pollutants at the time of emission, and what new compounds form subsequent to the photochemical activity of the atmosphere? 2. What is the physical state of the pollutant material—gaseous, gaseous-particulate (aerosol), solid-particulate (soot), or combinations of these? 3. Is the health effect related to the physical and chemical nature of the pollutant, or do allied factors enter, including, as studied by others, possible increased host susceptibility to upper and lower respiratory tract disease?* 4. Is the host response specific in terms of specific pollutants? 5. Do various pollutants act synergistically or in combination to produce a morbid response, since even in disasters the maximum allowable concentration of the toxic substances measured has with one exception never been reached?[7] 6. Is the effect solely one of addition of toxic substances to the atmosphere, or is there a secondary factor, such as interference with solarization, an item related to skin tumor morbidity and antirachitic activity?[8] Finally, is there a psychic effect, and, if so, how can it be measured?

Materials chosen to be studied were for the purpose of determining the effect of the atmospheric oxidants on appropriate animal species. While the oxidant effect is certainly not the only pollutant effect, chemical, engineering, and biological knowledge is most advanced in relation to it, and on this basis it was selected as the first property to be tested.

Subsequent to the selection of the oxidant effect as the initial test material and following a series of crude preliminary exposures in mice and rats, it became apparent that short-term experimental exposures to realistic or naturally occurring concentrations of oxidants would be of limited benefit. Changes produced would be so subtle that standard methods of laboratory measurement would be incapable of measuring the alterations in the biological systems chosen for study. A second variable that required delimitation was the time length of exposure to which animal species should be subjected. Preliminary observations indicated that the concentration percentage of pollutants was of greater significance than the exposure time.

* References 4 to 6.

Consistent with these observations, in our first experiments a factor of 10 was decided upon as being a satisfactory compromise in terms of producing demonstrable and perhaps accelerated changes in the test species while, on the other hand, still keeping the oxidant exposure sufficiently low so as to introduce little or no experimental artifact. Unless otherwise stated, the exposure concentration in our test

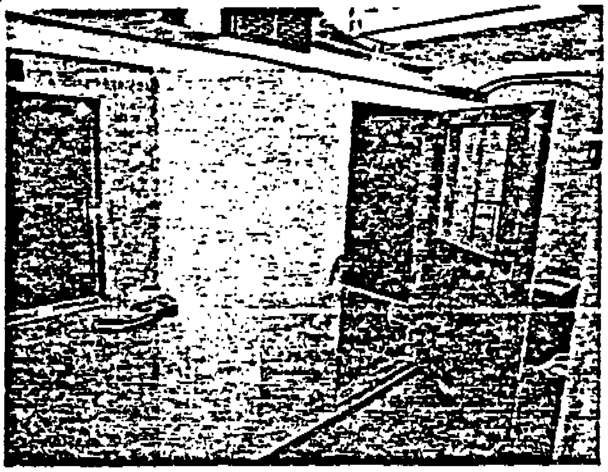

Fig. 1.—Two large chambers for large mammalian species studies; interior dimensions, 6 in. by 6 ft. 7 in. by 5 ft. 8 in.

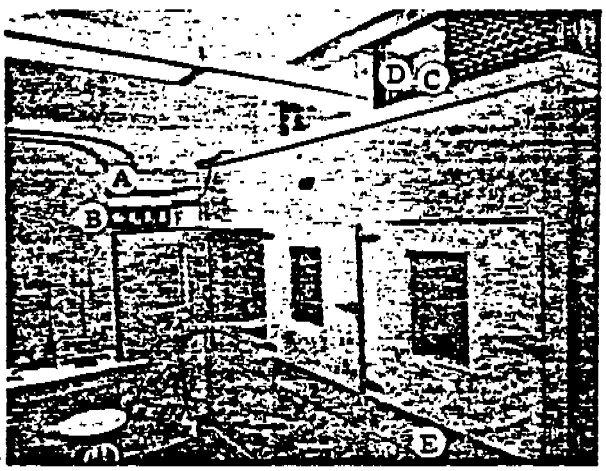

Fig. 2.—Three small chambers for medium-size mammalian species studies; interior dimensions, 2 ft. 6 in. by 2 ft. 6 in. by 3 ft. Switches at *A* for ultraviolet and fluorescent light energy sources for catalyzing photochemistry of artificial smog. Switches on control panel at *B* for Variac units controlling air flow through intake and exhaust air-circulating systems. *C*, lower half of intake filtering system, with Fiberglas and activated carbon filters. *D*, stainless steel neck above chambers, with intake lines for feeding pollutants into chambers. *E*, tap for sampling of chamber.

chambers measured 4 ppm total oxidant. This amount represented 10 times the total oxidant of 0.4 ppm measured in downtown Los Angeles at noon on a smoggy day.[8] Throughout the entire study it was repeatedly noted that additional tests, which are now under pursuit, would be necessary to further establish the equivalence of natural and synthetic smog.

Figures 1, 2, 3, and 4 show inhalation chambers, with associated control and analytical equipment.

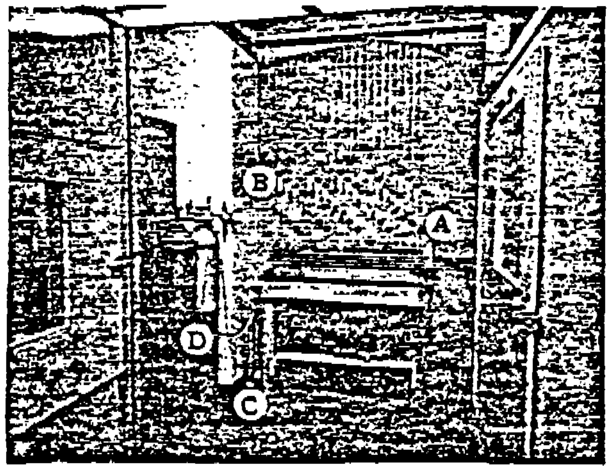

Fig. 3.—*A*, valve. *B*, manometer assemblies for control and measurement of pollutants prior to feeding into chambers. *C*, multiple ozonizers for conversion of oxygen into ozone. *D*, oxygen and nitrogen sources for hydrocarbon vaporization and ozone formation.

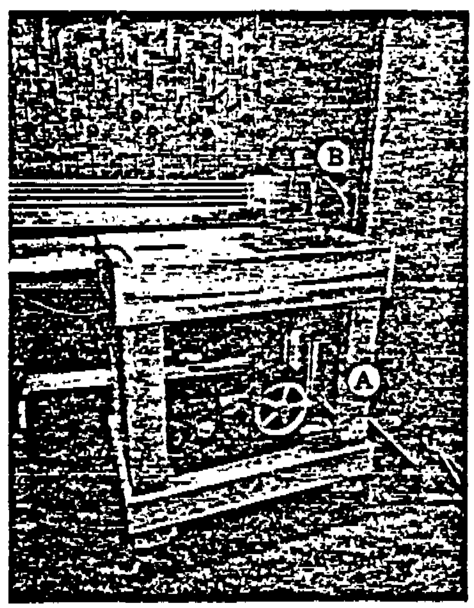

Fig. 4.—Close-up of valves and manometers. *A*, cart with vacuum pump for continuous or intermittent sampling of chambers. *B*, impingers with reagents for collection of pollutants.

Biological.—Biological investigation can be pursued in two fundamental physiological systems: in vitro and in vivo. The choice of the individual system must depend upon technical feasibility and the particular function or physiological activity being investigated.

MEDICAL INVESTIGATION OF AIR POLLUTION

In vitro studies are primarily concerned with the establishment of basic changes on the biochemical level so that clues as to the direction of in vivo studies may be obtained. All experimental studies were planned on the basis of increasing complexity of test systems, and the biochemical, physiologic, and pathologic disciplines were utilized in their pursuit. Chemical studies were chosen for the initial experiments for the twofold purpose of, first, determining the degree of smog sensitivity of physiological compounds and, second, establishing the need for air-conditioned laboratories so that the natural atmosphere would introduce no artifact into the data.

In vitro studies were made on amino acids, vitamins, nonprotein hormones, purines and pyrimidine compounds, porphyrin group compounds, blood and blood pigment substances, serum proteins, and enzyme systems.

In vivo studies were undertaken with use of mice, rabbits, and dogs in intact animal systems. Studies included (1) protein and enzyme studies to determine pollutant effects on plasma proteins and on enzyme and enzyme systems in intact animals; (2) respiratory physiology studies utilizing pulmonary function measurements and blood gas analyses in experimental species with and without decreased cardiorespiratory reserve; (3) amino acid studies to indicate protein structure changes through the knowledge of their fate; (4) hemoglobin studies to determine pollutant effects on oxyhemoglobin and nitric oxide hemoglobin, methemoglobin, and sulfhemoglobin formation; (5) pathologic studies to demonstrate morphologic changes in the respiratory tract and other organ systems, and (6) electrolyte studies to measure changes in blood concentration and excretion.

In addition to the already mentioned considerations in the accumulation of biological data, the problem of extrapolation of the findings to human circumstances must be constantly kept in mind. This necessitates the introduction of another, and perhaps critical, variable into the problem of evaluating experimental data. This problem of host response appears to be of fundamental significance when one considers the epidemiologic observations made in relation to air-pollution disaster episodes and the phenomenon of lung cancer increase.

The repeated observation that morbidity and mortality are primarily in persons with antecedent cardiorespiratory disease during periods of abnormal air-pollution concentration necessitates the reproduction on the experimental level of this condition. Artificial interference with respiratory physiology, cardiac function, and general physical status must be the basis for investigating one group of animals. Analogous and simultaneous experiments on identical species with normal or uninterfered with biological systems must also be undertaken. Finally, controls, as in all experimental studies, must discipline the investigation.

In chronic studies, including the carcinogenic ones, epidemiologic observations again indicate the need for certain additional laboratory controls. 1. Is sexual difference in the frequency of lung cancer which has been observed in humans applicable to experimental species? 2. Is the difference between the daily environments of men and women capable of laboratory investigation? 3. Is the difference between urban and rural lung cancer frequency also possible of delineation in laboratory studies? And, finally, can laboratory studies be oriented in the direction of duplicating the human experience in relation to lung cancer, both by using inhalation methods of experimentation and by aiming the research at the same target tissue, the lung?

CONCLUSIONS AND SUMMARY

The laboratory investigation of the biological or medical effects of air pollution presents three separate major problems. The first is concerned with the qualitative and quantitative reproduction of air pollutants in the laboratory as a readily available tool. This requires basic knowledge as to the engineering, chemical, and physical aspects of pollutant sources. In addition, the creation of new compounds in the atmosphere subsequent to photochemical activity must also be studied. The second problem is concerned with the choice of biological systems. Ideally, in vitro studies should direct toward the more complicated in vivo ones, and individual experiments should progress on the basis of increasing complexity. No organ or organ system should be free from suspicion in terms of morbid response to air pollution. The third major problem is concerned with the duplication of human experience in laboratory circumstances. In the acute phase this requires the paralleling of the various states of health, or more appropriately ill health, as they exist in the general population. This may be critical in view of morbidity and mortality observations during the acute episodes of air pollution. The significance of carcinogenic studies is directly related to the ability to explain the epidemiological and pathological observations made in relation to lung cancer. Specifically, the histopathological pattern of the cancer, the organ site, the sexual difference in frequency, and the urban and rural difference in incidence are some of the problems to which the laboratory might provide the answers.

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