BIOCHEMICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS

Edited by S. D. LEE

BIOCHEMICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS

edited by

S. D. LEE

Health Effects Research Laboratory
Environmental Research Center
U.S. Environmental Protection Agency
Cincinnati, Ohio

with the assistance of

Bruce Peirano

ANN ARBOR SCIENCE
PUBLISHERS INC
P.O. BOX 1425 • ANN ARBOR, MICH. 48106

230 Collingwood, P. O. Box 1425, Ann Arbor, Michigan 48106

Library of Congress Catalog Card No. 76-46022
ISBN 0-250-40143-6

Manufactured in the United States of America

PREFACE

This volume derived from a symposium organized to emphasize the value of understanding the biochemical effects of environmental pollutants. Detection of early biochemical lesions that are related to subsequent changes in structure and physiology would be useful as early signs of environmental hazards that produce disease in humans. Too often in the past such hazards have been defined only after outbreaks of human cases have occurred. Epidemiologic studies identifying cadmium, methyl mercury, asbestos and kepone are only recent and publicized examples of the after-the-fact approach to environmental protection.

Perhaps even more important in the long term, basic understanding of the mechanisms by which environmental chemicals produce their effects appears as the only rational basis for predicting the hazards associated with the mixtures of chemicals encountered in the real world. Empirical testing of all possible pollutant combinations is decidedly unachievable because of the sheer volume and expense of such an enterprise. Consequently, prediction based on detailed knowledge of the biochemical and pharmacodynamic properties of individual chemicals appears to be the only viable alternative for making regulatory decisions.

Trace metal and oxidant pollutant toxicology were chosen as the general areas of discussion because they have been researched sufficiently to begin their assessment in light of the general aims stated above. Incorporation of these separate fields results in a certain degree of discontinuity. However, all the presentations made have directly or indirectly assessed these major questions–some have emphasized the interaction of nutritional factors in modifying the effects of toxic substances, and others deal with the multiplicity of mechanisms often possible which interfere with particular systems of even single enzymes. Finally, specific interactions between environmental chemicals have been demonstrated. A comprehensive classification of chemical effects of predictive value remains illusive. The main reason is that most research emphasizes purely

empirical goals with little regard for the general knowledge which should arise out of any toxicologic study. These goals are not at all contradictory. They simply involve formation of specific and testable hypotheses which go beyond the overly simplistic question of "effect" and "no effect" concentrations of chemicals. Such an approach would serve to channel research in productive directions, avoiding the expensive comprehensive approach more aptly called the "shotgun rationale" which seems to guide a major share of efforts in environmental health.

Responsibility for the scientific content of each contribution lies with the authors, although the authors cooperated in a considerable effort toward editorial clarification.

Sincere gratitude is extended to Dr. R. John Garner, Director, Health Effects Research Laboratory, EPA, Cincinnati, for his support. We are indebted for the time and efforts of Drs. Donald Tierney, Harold Petering, Milos Chvapil, Vincent Finelli, and R. S. Bhatnagar for the organization of the symposium and to the individual contributors who made the entire effort a success. A special acknowledgment is necessary for the tireless assistance provided by Mr. Bruce Peirano and Mrs. Carol Haynes.

Si Duk Lee
Cincinnati, Ohio

CONTENTS

CHAPTER 1

BIOCHEMICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS

KEYNOTE ADDRESS

D. S. Barth

Deputy Assistant Administrator for Health
Health and Ecological Effects
U.S. Environmental Protection Agency
Washington, D.C. 20460

INTRODUCTION

In order to put the subject of this Symposium into perspective, I deem it necessary to spend some time discussing the objective of EPA, how this objective is being attained, and how outputs from health effects research contribute to the attainment of this objective. I will then discuss the importance of biochemical effects research in relation to health effects research in general.

EPA'S OBJECTIVES

The subjects dealt with by EPA include air pollution, water pollution, pesticides, solid waste management, radiation, noise and toxic substances. In brief, the objective of EPA is to abate or control environmental pollution to socially acceptable levels. The role of research and development then is to provide a body of research information sufficient to enable an informed judgment to be made with regard to acceptable levels for various environmental pollutants.

The laws that EPA must implement are many and varied. However, the intent of Congress in each law is aimed at the protection and enhancement of the environment. This implies that required controls are generally designed to abate adverse effects on health or welfare to

acceptable levels or to prevent the occurrence of new adverse effects. In all instances health effects are deemed to be of primary importance, with welfare effects being secondary but still of major concern.

EPA'S OFFICE OF RESEARCH AND DEVELOPMENT PROGRAMS TO MEET OBJECTIVES

The research and development program of EPA is generally concerned with the following subject areas:

effects
environmental exposure levels
predictive models linking source emissions to exposure levels
control technology

Effects research includes development of exposure-effect relationships for selected environmental pollutants, acting singly or in combination, on selected populations of receptors for both health and welfare effects.

The documentation of environmental exposure levels is essentially a monitoring task. Such exposure monitoring data are necessary to determine where and to what extent environmental exposure levels exceed acceptable values and to measure the efficacy of control programs as they are implemented. Validated predictive models linking source emissions to exposure levels are required to design the most cost-effective control plans for source emissions to reduce exposure levels to acceptable values. Control technology must be available to control major emission sources adequately. In many cases this requires extensive research and development and demonstration programs.

Let us now consider in somewhat more detail the outputs required from our health effects research programs and the research methods and approaches used to obtain those outputs. As already mentioned, we seek exposure-effect relationships for selected pollutants, acting singly or in combination, on selected populations at risk.

Principal factors to be considered in the selection of pollutants for study include:

our present state of knowledge for both regulated and unregulated pollutants
known or suspected seriousness of adverse effects from over-exposure
availability of adequate measurement methods
size of the populations at risk and estimates of exposure levels
occupational health experience with pollutants under consideration

Principal factors to be considered in the selection of populations for study include:

most sensitive populations at risk
higher exposure levels for most sensitive populations

likelihood of the presence of contributing factors not related to environmental pollutant(s).

Approaches used to perform health effects research in EPA include epidemiological, toxicological and clinical studies. Whenever possible, all three approaches are used in a coordinated fashion. Biochemical studies may be included in any or all of the three approaches.

Our recently implemented program to assess the contribution of environmental carcinogens to cancer incidence in the general population is an example of the meshing of these areas in a comprehensive study. The initial thrust of this program will include media transport assessment, inter- and intramedia transformation, measurement methodology, exposure monitoring, dose assessment, and retrospective estimation of exposure, all conducted under a rigorous quality assurance program in areas of high and low cancer incidence. After this initial phase, the coordinated data base will generate a requirement for targeted epidemiological and toxicological studies. Finally, the information will provide values for the construction and validation of a predictive model.

ROLE OF BIOCHEMICAL EFFECTS RESEARCH IN ORD PROGRAM

A distinction can be made in biochemical effects research between "effects monitoring" and "health effects." The former is a requirement for the final stages of environmental exposure monitoring, such as exposure/dose assessment. The latter involves establishing a meaningful relationship between a biochemical change and the health or well-being of the exposed population.

Toxicological lethality studies have long been used to evaluate the hazards of various chemicals; however, such methods are relatively gross because of the comparatively large doses required to produce observable effects within the short life spans of the experimental animals. It is here that the study of biochemical effects may offer an advantage. Such effects undoubtedly precede such end points as the LD_{50} and, if thoroughly understood, not only may explain the mechanism of the hazard but also may indicate methods of control or reversal. These considerations suggest that possible hazards may be identified and their effects estimated long before the results of chronic toxicity or epidemiological studies are available. This would be particularly true for carcinogenic chemicals that produce their end effects only after a long latent period and for those, such as lead, that may accumulate slowly to an end-effect level with continued exposure.

Some results along this line are already appearing; for example, the relationship among blood-lead levels, ALA-D, and urinary homovanillic acid concentration and the somewhat tenuous relationship between mutagenesis and carcinogenesis that is the basis for the proposed use of the bacterial mutagenesis test for detection of carcinogens. To expand on these somewhat, if the indication of nerve damage suggested by the biochemical detection of increased homovanillic acid excretion can be confirmed, then an early indication of harmful effects may be possible so that control can be established before permanent harm ensues. Erythrocyte ALA-D, on the other hand serves as a convenient "effect" for relating environmental exposure to dose assessment. The subject of mutagenesis was addressed in the December 1975 Nobel lecture by Dulbecco, who advocated widespread use of bacterial mutagenicity tests before releasing any new compound to the public. The feasibility of such a program is strengthened by the finding that most of the commonly available substances are not promutagens.

The foregoing are examples of biochemical effects used in different applications. The sensitivity, and even specificity, of such tests hold great promise for the future. If developed to full potential, biochemical changes related to the assessment of human health and welfare effects would significantly aid the EPA in fulfilling its mandate to protect and enhance the environment.

FUTURE CHALLENGES

Some current and future problems of major concern to EPA's health effects research programs include:

development of suitable animal models for extrapolation to humans

development of adequate screening tests, *in vitro* or *in vivo*, to estimate toxic properties of environmental pollutants

development of methods for determining effects in humans or experimental animals of long-term, low-level exposures

development of methods for determining varying effects of different averaging times for different exposures

development of methods for measuring and interpreting physiological or biochemical changes occurring as precursors to disease

development of methods for biological monitoring to quantitate exposure levels

development of personal exposure meters to improve our ability to assess exposure to air pollutants

development of biochemical exposure indicators to assess exposure by any route.

This symposium was convened to address some of the problems I have enumerated, and to assess current progress in the area of biochemical change as related to effects of environmental pollutants. We can expect much mutual education from the discussions.

CHAPTER 2

CELLULAR APPROACHES TO THE STUDY OF ENVIRONMENTAL POLLUTANTS

N. A. Elson and R. G. Crystal

Pulmonary Branch
National Heart, Lung, and Blood Institute
Building 10, Room 6N260
Bethesda, Maryland 20014

INTRODUCTION

Epidemiologic and animal studies strongly suggest that airborne pollutants are commonly encountered in the urban environment in concentrations which are toxic to the lungs.[1-3] Detailed studies have resulted in the identification of those pollutants which cause injury and the development of guidelines relating concentration, form and time of exposure to the relative toxicity for each pollutant.[2] With this kind of animal data available as a groundwork, workers in the respiratory pollutant field are turning their attention toward validating animal toxicity studies by evaluating humans exposed to airborne pollutants; identifying the mechanisms by which airborne pollutants cause lung injury; and determining how pollutant-related pulmonary injury can be circumvented or treated.[4]

There are formidable obstacles to the solution of these problems. Direct biopsy evaluation of human lung under controlled conditions is impossible, and so to validate animal toxicity studies, only safe, and hence indirect, methodologies can be used. Even in animal studies, the task of identifying the pathogenic mechanisms is complex, since the lungs of animals exposed to pollutants show the results of multiple injuries. This yields a montage of the secondary effects of inflammation, clearance

mechanisms and repair processes, occurring simultaneously or in overlapping sequence to the primary toxic effect of the pollutant. Thus, to expand our understanding of the effects of airborne pollutants, it is necessary to develop new methodologies on two fronts: (1) safe, non-invasive methods to evaluate the effect of pollutants on man; and (2) approaches which will simplify the evaluation of the mechanisms of pollutant-related lung injury.

To accomplish these goals, most attention is being focused on the cells of the lung. While it is conceivable that environmental pollutants directly affect the pulmonary extracellular matrix, most available evidence suggests the earliest injury is at the cellular level.[1] By developing methodologies to evaluate the cells of the lung, we can gain insight into how the lung responds to inhaled pollutants, and thus determine safe limits of exposure and pathogenic mechanisms of pollutant-related lung injury. The solutions to these problems will eventually form the basis of a rational approach to prevention, diagnosis and treatment of pollutant-related respiratory disease.

INDIRECT STUDIES OF LUNG CELLS

While population studies implicate the toxicity of airborne pollutants on the human lung, it is only through carefully controlled exposure chamber studies that specific information can be developed. As discussed at this conference, short exposure to low concentrations of ozone can result in functional abnormalities manifested by mild obstruction to airflow.[5-6] Since this phenomenon is presumably secondary to the primary effects of ozone on the constituent cells of the lung*, evaluation of lung cells in these individuals would provide several important pieces of information: (1) which cells are involved; (2) how the pollutant affects each cell; and (3) the mildest exposures which cause cellular dysfunction. The last is of particular importance, since it is probable that cellular dysfunction precedes physiologic dysfunction. Although our laboratory has not specifically studied the effects of airborne pollutants in humans, we have utilized several methods which yield information on the status of lung cells in man, particularly those cells concerned with inflammatory and immune mechanisms.

---

*"Lung cells" will be used to refer to any cell comprising the parenchyma, airways or blood vessels of lung, plus blood-derived cells that may reside in lung.[7-9] In the normal individual, the latter refers to monocytes (or their daughter macrophages) and lymphocytes. In diseased lungs, neutrophils, eosinophils and/or basophils may also be present.

Bronchoalveolar Lavage

The fiberoptic bronchoscope has greatly expanded acess to the lung.[10] Besides its use in diagnosing tumors and infection, this instrument can be used to sample the cells and fluids which bathe the bronchoalveolar epithelial surface. This procedure, termed bronchoalveolar lavage, is simple, safe and rapid and has been performed on many normal volunteers without complications (Figure 2.1).[11,12] The bronchoscope is positioned in a subsegmental airway of the lingula and 100 ml of saline

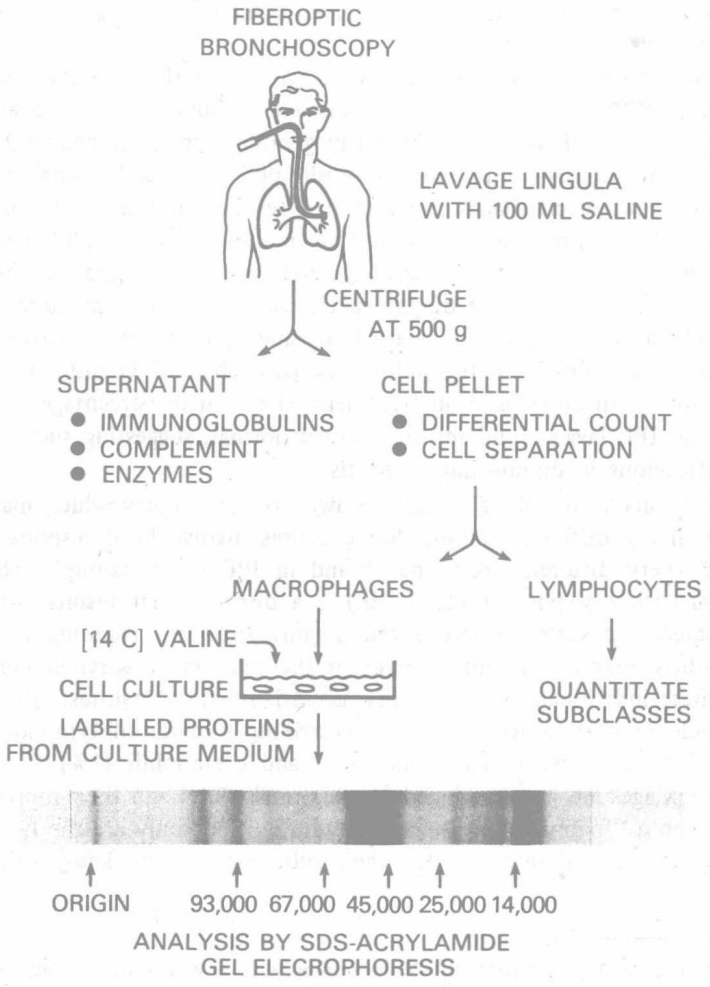

Figure 2.1. Schematic of the procedure of bronchoalveolar lavage and subsequent analysis of lavage cells and proteins.