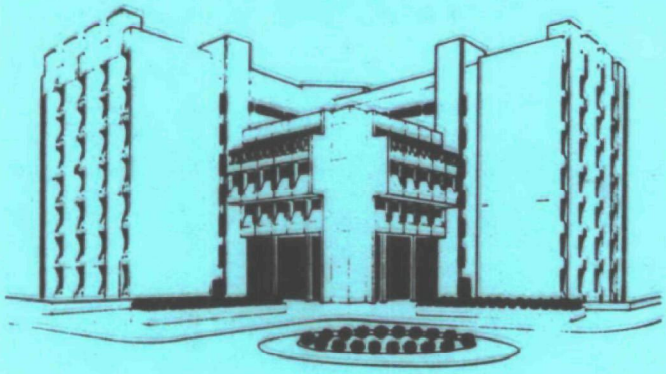
BIOCHEMICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS: AN EPA SYMPOSIUM

ENVIRONMENTAL RESEARCH CENTER

(Adjacent to University of Cincinnati Campus)

MAY 31 - JUNE 3, 1976



Sponsored By:

Health Effects Research Laboratory
Environmental Research Center
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

PURPOSE OF THE SYMPOSIUM

This symposium is being 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 associated with disease would be useful for early signs of hazards to health in human populations. More importantly in the long term, this approach appears as the only rational basis for making concrete decisions and predicting the hazards of the mixtures of chemicals encountered in the real world environment. Testing of all possible pollutant combinations empirically is decidedly unachievable because of the sheer volume and expense of such an enterprise. Consequently, prediction based upon detailed knowledge of the biochemical and pharmacodynamic properties of individual chemicals appears as the only viable alternative. The topics of the present symposium deal with two areas in which a large volume of research has been done. Perhaps a framework for prediction interactions may be discernible at this time. If not, it is hoped that this meeting will stimulate the participants and others concerned with the effects of environmental chemicals on health to focus future research in the direction of developing the information required for predictive models.

SYMPOSIUM COMMITTEE

Dr. R. John Garner — General Chairman

Organizing Committee

S. D. Lee, Chairman
R. Bull
H. Petering

Program Committee

J. F. Stara, Chairman
V. F. Finelli
D. Tierney - UCLA
R. Bhatnager - UCSF

MONDAY, MAY 31, 1976

1:00 - 7:00 p.m.

Registration

(Stouffer Inn)

TUESDAY, June 1, 1976

8:30 am Welcome and Introductory Remarks
Dr. R. John Garner

8:45 am Keynote
Dr. Delbert Barth

SESSION I: BIOCHEMICAL EFFECTS OF GASEOUS POLLUTANTS

Co-chairman: Dr. Tierney
Co-chairman: Dr. Garner

9:00 - 9:30 am Biochemical Effects of Oxygen Toxicity, *Dr. D. Tierney*

9:30 - 10:00 am Cell Renewal and Adaptation in Rat Lungs Exposed to Ozone, *Dr. Michael Evans* – Stanford R.I.

10:00 - 10:15 am COFFEE BREAK (CAFETERIA)

10:15 - 10:45 am Nucleic Acid Metabolism in Normal and Damaged Lung, *Dr. H. Witschi*

10:45 - 11:15 am Role of Superoxide in Oxidant Induced Pulmonary Fibrosis, *Dr. R. Bhatnager*

11:15 - 11:45 am Open Discussion

11:45 - 1:00 pm LUNCH

1:00 - 1:30 pm Biochemical Effects of Environmental Oxidant Pollutants in Animal Lungs, *Dr. M. Mustafa* UCLA

1:30 - 2:00 pm Effects of Air Pollutant Oxidants on Biological Membranes, *Dr J. B. Mudd* UC R.

2:00 - 2:30 pm Membrane Structure, Bulk Phase Lipid Properties and the Effects of Small Molecules on Membrane Functions, *Dr. G. Rouser* City of Hope –

2:30 - 2:45 pm COFFEE BREAK (CAFETERIA)

2:45 - 3:15 pm Ozone and Airway, Hyperirritability, *Dr. Lu-Yuan Lee* UCSF

3:15 - 3:45 pm Cellular Events in Inflammation, *Dr. Yi Han Chang* UCLA

3:45 - 4:15 pm Human Biochemical Effects of Gaseous Pollutants, *Dr. R. Buckley* USC

4:15 - 4:45 pm Open Discussion

WEDNESDAY, JUNE 2, 1976

SESSION I (continued)

9:00 - 9:30 am	Effects of Sulfuric Acid Aerosol on Respiratory System Morphology, *Dr. W. Tyler* UC Davis
9:30 - 10:00 am	In Vivo and In Vitro Effects on Sulfur Dioxide Upon Biochemical and Immunological Parameters, *Dr. E. Gause*
10:00 - 10:15 am	COFFEE BREAK (CAFETERIA)
10:15 - 10:45 am	Sulfur Dioxide: A Review of Its Reactions with Biomolecules, *Dr. D. Petering*
10:45 - 11:15 am	Biological Origin and Metabolism of So_2, *Dr. K. Rajogopalan*
11:15 - 11:45 am	Open Discussion
11:45 - 1:30 pm	LUNCH

SESSION II: BIOCHEMICAL EFFECTS OF HEAVY METALS

Co-chairman: Dr. Chvapil
Co-chairman: Dr. Petering

1:30 - 2:00 pm	Cellular Mechanisms of Lung Fibrosis, *Dr. M. Chvapil*
2:00 - 2:30 pm	Cellular Approaches to the Study of Environmental Pollutants, *Dr. R. Crystal*
2:30 - 2:45 pm	COFFEE BREAK (CAFETERIA)
2:45 - 3:15 pm	Biochemical Interactions of Environmental Metal Contaminants with Lung Connective Tissues, *Dr. M. Hussain* UCSF
3:15 - 3:45 pm	Environmental Effects on the Biosythesis of Lung Elastic Tissue, *Dr. J. Foster*
3:45 - 4:15 pm	Open Discussion
6:30 pm	SOCIAL HOUR
7:30 pm	BANQUET (University of Cincinnati Faculty Club)

THURSDAY, JUNE 3, 1976

SESSION II (continued)

8:30 - 9:00 am	Role of Nutrition in Heavy Metal Toxicity, *Dr. H. Petering*
9:00 - 9:30 am	ALA-D in Lead Exposure, *Dr. V. Finelli*
9:30 - 9:45 am	COFFEE BREAK (CAFETERIA)
9:45 - 10:15 am	Bioinorganic Chemical Reactions in the Environment, *Dr. J. Wood*
10:15 0 10:45 am	Effects of Heavy Metals on Isolated Mitochondria, *Dr. G. Brierley*
10:45 - 11:15 am	Metabolic Interactions of Selenium with Heavy Metals, *Dr. R. Rimerman*
11:15 - 11:45 am	Open Discussion
11:45 - 1:00 pm	LUNCH
1:00 - 1:30 pm	Platelet Metabolism in the Study of Molybdenum and Other Trace Substances, *Dr. C. Solomons*
1:30 - 2:00 pm	Neurotransmitter Mechanisms and Inorganic Lead Poisoning, *Dr. A Goldberg*
2:00 - 2:15 pm	COFFEE BREAK (CAFETERIA)
2:15 - 2:45 pm	Evaluation of Animal Models Used to Study Lead Effects on Neurochemistry and Behavior, *Dr. I. Michaelson*
2:45 - 3:15 pm	Effects of Trace Metals and Their Derivatives on the Control of Brain Energy Metabolism, *Dr. R. Bull*
3:15 - 3:45 pm	Open Discussion
3:45 pm	Epilog, *Dr. R. John Garner*

BIOCHEMICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS

KEYNOTE ADDRESS

by D. S. Barth

I. 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.

II. 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 judgement to be made with regard to acceptable levels for various environmental pollutants.

The laws which 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.

III. EPA'S ORD 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, development and demonstration programs.

Let us now consider in somewhat more detail what outputs are required from our health effects research programs and what research methods and approaches are used to obtain those outputs. As I 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 helath effects research in EPA include epidemiologic, toxicologic 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 intra-media 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 toxicologic studies. Finally, the information will provide values for the construction and validation of a predictive model.

IV. 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,

i.e. 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 LD50 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 which produce their end effect only after a long latent period and for those, such as lead, which 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 which 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.

V. 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

VI. EPILOGUE

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 scheduled during the next three days.

ABSTRACTS

OXYGEN TOXICITY AND LUNG BIOCHEMISTRY

The phenomenon of adaptation or tolerance of lungs to increased partial pressures of oxygen led us to consider that a metabolic adaptation may have occurred. We therefore, compared some aspects of lung metabolism in control and adapted animals. Initially we searched for changes in pathways which might be mechanisms to protect against oxidants. We found, for instance, changes in activity of glucose-6-phosphate dehydrogenase and Crapo and I observed increases of superoxide-dismutase. We were unable to detect similar changes in a strain of mice which did not adapt but did have inflammatory changes from the oxygen exposure. Although these observations supported the concept that these enzymes might play an important role in the adaptation, the increased activity of these enzymes may be coincidental and evidence from other investigators indicate that the change of enzyme activity may be a nonspecific response to injury.

Two chemicals alter the sensitivity to oxygen toxicity. Alpha-napthyl-thiourea (ANTU) when given intraperitoneally to rats leads to relative tolerance for oxygen one week later. ANTU also produces changes of glucose-6-phosphate dehydrogenase similar to those from 85% O_2 for 7-days. ANTU is reported to have a primary effect upon lung capillaries with little involvement of epithelial cells. The pesticide paraquat, when injected into rats, increases the sensitivity to oxygen toxicity as

demonstrated by Fisher and Clements. Paraquat also leads to a markedly (4-6 fold) increased activity of the pentose pathway as determined by $^{14}CO_2$ production from glucose labelled in the first or sixth position. Paraquat is thought to generate superoxide and its effect may, therefore, be similar to oxygen toxicity. The increased activity of the pentose pathway with paraquat may be related to reduction of oxidants or peroxides through glutathione. However, the toxic effects of paraquat as determined by decreasing QO_2 of tissue slices are not different when glucose is present or absent.

Lipid synthesis in rat lungs is altered by oxygen toxicity and during the recovery phase in air the content of saturated phosphatidylcholine nearly doubles. This increased content may relate to pulmonary surfactant and increased numbers of type II cells.

Oxygen injury to the lung cells may have a similar mechanism to other oxidant injuries including ozone and nitrogen dioxide. However, the high PO_2 with oxygen breathing extends to all parts of the lung and the distribution of injury may be very different from inhalation of low concentrations of gases (ozone and nitrogen dioxide) which may be lost from the inhaled gas as they impact against the wall of the airway.

Abstract

CELL RENEWAL AND ADAPTATION IN RAT LUNGS EXPOSED TO OZONE

by

Michael J. Evans, Ph.D.

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This research was undertaken to study the early effects of low levels of ozone on cell renewal in the lungs of rats. To accomplish this, male rats were exposed to ozone for up to 8 days. Dividing cells were labeled with tritiated thymidine (^{3}H-TdR) and studied with autoradiographic techniques in the light microscope. The results showed that all labeled cells increased and then decreased to near control levels within 4 days. Type 2 cells showed the largest change in labeling index. Because the labeling indexes decreased by the fourth day despite continuous exposure and because no further injury occurred, it was assumed that the tissue had become tolerant to that concentration of O_3. To test the degree of tolerance, groups of animals adapted to O_3 were exposed to higher concentrations of O_3, and the labeling indexes of Type 2 cells were studied. These studies showed that tolerance to the initial concentration of O_3 did not ensure total protection to rats against reexposure to higher concentrations of O_3.

To determine which cells were being injured on reexposure to O_3, another experiment was performed. Rats were exposed to O_3 for 2 days, labeled with ^{3}H-TdR, and allowed to recover for 4 days. Because Type 2 cells transform into Type 1 cells within 3 days under these conditions, this treatment left labeled Type 2 and Type 1 cells in the alveoli. These "adapted" rats were then reexposed to higher concentrations of O_3 and the number of labeled Type 2 and Type 1 cells were determined. Preliminary results show that labeled Type 1 cells were not reinjured on reexposure to O_3, indicating that they are adapted cells in the alveolar epithelium.

NUCLEIC ACID METABOLISM IN NORMAL AND DAMAGED LUNG

Hanspeter Witschi

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Pulmonary RNA synthesis can be evaluated in vivo by injecting radiolabelled uridine or orotic acid and determining the specific activity of total pulmonary RNA. In rats, pulmonary RNA synthesis proceeds at about the same rate as in liver, whereas in hamster lung it is less. The kinetic constants of enzymes involved in pyrimidine biosynthesis are comparable between lung and liver. In comparing RNA synthesis in normal and chemically damaged lung, variability in precursor pool size has to be taken into account. It may be possible to overcome this problem by measuring incorporation of uridine or orotic acid into RNA in vitro in lung slices or minced lung tissue; incorporation rates are linear up to 40 min. incubation time.

Pulmonary DNA synthesis may be measured in vivo by following the incorporation of thymidine-^{14}C into DNA. In normal lung, incorporation rates are very low. However, in lungs injured by several chemicals an intense proliferation of type II alveolar cells repairs the damage. This repair process can easily be followed by measuring in vivo DNA synthesis. Thymidine kinase, an enzyme found in a high-speed supernatant prepared from lung homogenates may serve as another biochemical indicator of cell proliferation in lung,

whereas DNA polymerase is much less suitable.

Proliferation of type II alveolar cells may be produced by treating mice with the antioxidant butylated hydroxytoluene (BHT; 250-500 mg/kg i.p.). Dose-effect and time-effect relationships have been quantitated by measuring *in vivo* DNA synthesis or thymidine kinase. Exposure to 100% oxygen inhibits DNA synthesis in lung. Oxidants do therefore not only produce lung damage per se, but will also compromise the following repair of the alveolar epithelium.

ROLE OF SUPEROXIDE IN OXIDANT INDUCED PULMONARY FIBROSIS

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Oxidants, e.g. nitrogen oxides and O_3, are a health hazard of increasing significance. Their primary target is the lungs which undergo functional and morphological deterioration, the severity of which depends on the extent of exposure. A major outcome of oxidant injury is interstitial thickening in the lungs involving increased connective tissue components. Exposure to oxidants results in chemical injury which elicits a reparative response. Inflammation and repair culminate in tissue alterations which hamper function and lead to proliferation of injury and repair resulting in progressively increasing deposition of connective tissue. Exposure to oxidants results in inflammation and marked increases in parameters of collagen synthesis. Interaction of oxidants (O_2,O_3) with physiological systems generates the superoxide free radical (O_2^-) and O_2 is also a major reactive species in inflammation. Symptoms of pulmonary injury are alleviated by the enzyme superoxide dismutase (SOD) which is also induced in exposed tissues as a protective response and by several anti-oxidants. Recent studies in our laboratory have shown that O_2^- is a component of the crucial hydroxylation steps in collagen synthesis and it also induces enhanced collagen polypeptide synthesis as well as prolyl hydroxylase in cells in culture. Thus it appears that the fibrogenic effect of oxidant gases in lungs may be mediated through O_2^- radicals. Because O_2 is consumed during collagen synthesis, this may be a protective mechanism against O_2^- toxicity, although excess collagen deposition may have deleterious effects on pulmonary function. Supported by EPA Contract #68-03-2005 and NIH Grant De-03861.

BIOCHEMICAL EFFECTS OF ENVIRONMENTAL OXIDANT POLLUTANTS IN ANIMAL LUNGS.

M.G. Mustafa, A.D. Hacker, J.J. Ospital and S.D. Lee. University of California, Los Angeles, California 90024 and Environmental Protection Agency, Cincinnati, Ohio 45268

Exposure to ozone or nitrogen dioxide arising from photochemical smog or from industrial or occupational environments may cause lung cellular injury and metabolic alterations. Although several laboratories have studied enzymatic or other biochemical changes in the lung due to ozone or nitrogen dioxide exposure, the data generated are rather sparse. The purpose of this study is to establish a general pattern of metabolic changes in the lung that result from exposure to oxidant pollutants. Animal exposures have been conducted with ozone at concentrations below 1 ppm (simulating ambient conditions in photochemical smog) or to nitrogen dioxide at 5 ppm (the Threshold Limit Value). The metabolic parameters studied include oxygen consumption, glucose utilization, pyruvate and lactate production, and protein and lipid biosynthesis in lung slices, substrate utilization in lung homogenate, and marker enzyme activities in subcellular fractions (viz., mitochondrial succinate oxidase, succinate-cytochrome c reductase; microsomal NADH- and NADPH-cytochrome c reductases; and cytosolic glucose-6-phosphate dehydrogenase, glutathione reductase, disulfide reductase, glutathione peroxidase and glutathione-disulfide transhydrogenase). Results shown below are from ozone exposures, but similar trends were obtained from limited exposures conducted using nitrogen dioxide. As judged from enzymatic activities (expressed as units per lung) lung metabolism may exhibit an initial depression (usually within 24 hours of exposure, reflecting an injury phase), but a subsequent elevation (coincident with the initiation of a repair phase) which peaks between 3 and 4 days of exposure. For exposures involving 0.8 ppm ozone, a 50-100% elevation of metabolic parameters has been observed. For lower levels of ozone (viz., 0.5 and 0.2 ppm) the changes in metabolic parameters are relatively small, exhibiting a fairly dose-dependent response. Ordinarily, no substantial changes in lung metabolism are observed for ozone exposure at 0.1 ppm. However, in animals maintained on a vitamin E-deficient diet, significant biochemical changes in the lung may result from exposure to 0.1 ppm ozone, and the changes may be considerably magnified for higher levels of ozone. Prolonged exposures to ozone (viz., 0.8 ppm) show that the biochemical changes in the lung, which reach the peak level between 3 and 4 days, remain elevated for as long as 50 days of exposure, but then decline almost to control level after 90 days of exposure. For lower levels of ozone (viz., 0.5 and 0.2 ppm) the changes are found to revert to control levels within 20 days of exposure. If the exposure is discontinued after a few days, the biochemical changes in recovering animals return to control levels within a week. It may be concluded that low-level oxidant exposures cause biochemical changes in the lung, the magnitude of which is dependent upon oxidant concentration as well as the nutritional status of animals with respect to dietary supplementation of antioxidants. These changes in enzymatic activities appear to be transient, i.e., they rapidly disappear after termination of an exposure, or slowly diminish during a prolonged exposure. (Supported in part by USEPA contract 68-03-2221 and USPHS grant HL17719; a part of the animal exposure was conducted at the California Primate Research Center, Davis, California).

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EFFECT OF AIR POLLUTANT OXIDANTS ON BIOLOGICAL MEMBRANES

Ozone has been shown to affect the permeability properties of plant cells, and its effect on alveolar macrophage may also be interpreted as an effect on the cellular membrane. Other consequences of ozone exposure show that there are responses distal to the membrane, but it is not clear that these are direct responses of ozone rather than responses to chemicals produced by primary reactions of ozone.

In model systems, it can be shown that ozone reacts with phospholipid vesicles and that the point of attack is at the double bonds of unsaturated fatty acids. Model systems also indicate that peroxyacetyl nitrate attacks at double bonds. As far as proteins are concerned, ozone attacks cysteine, tryptophan, methionine, histidine, tyrosine, phenylalanine and cystine. Peroxyacetyl nitrate attacks only cysteine and methionine.

When ozone and peroxyacetyl nitrate in polluted air reach the plasma membrane of a lung alveolar cell or the mesophyll cell of a leaf, is the protein or the lipid component of the membrane affected first? Can the oxidants penetrate the membrane unchanged and affect the cell contents directly? Our recent evidence shows that the external proteins of the membrane are readily attacked by ozone, and that there are distinct effects on proteins at the inner surface of the membrane before any effects on lipid can be detected.

Membrane Structure, Bulk Phase Lipid Properties, and the Effects of Small Molecules on Membrane Functions

by George Rouser

In membranes, studies with spin labels and other methods have shown that some of the lipid binds directly to protein on one side and to other lipid molecules on the other to form a lipid compartment in which most of the lipid has bulk phase properties closely similar to those of lipid extracted from the membrane. Many of the membrane proteins have enzymatic activities or transport functions that are entirely dependent upon their being associated with phospholipid having a particular type of polar group and acyl chains of the proper length and degree of unsaturation. These specific requirements are dictated by the specificity of lipid-protein interaction, and the requirement that lipids be in the fluid state at body temperature. This delicate balance of factors can be upset by the entrance of small molecules into the lipid phase. A striking example is provided by the inert gas Xenon that is a potent general anesthetic that acts by entering membranes and blocking conduction. Xenon enters "free" space in membranes but does not interact chemically. Entrance into the "free" space appears to be relatively specific for molecules of the proper size since other inert gases are much less effective. A comparison of the types of molecules that are effective general anesthetics indicates the existence of both polar and non-polar "free" or potentially free spaces in membranes. Despite its importance, systematic exploration of the effects on enzyme activites and transport processes of small molecules that do not react with membranes to form covalent bonds has not been undertaken.

The way in which small molecules that can react with double bonds of lipid carbon chains may influence membrane functions can be understood, at least in part, from the bulk phase lipid properties with change in temperature.

Arrhenius plots of enzyme and transport functions show abrupt changes at characteristic temperatures determined by the type of lipid. Some of the abrupt changes in rate are due to transition of fluid lipid to a more visious fluid rather than the solid state. It is thus possible that small, reactive molecules that can link lipid molecules may cause a change in activity similar to that produced by lowering temperature to force cooperative movement. The type of change can be expected to depend upon the size and shape of the perturbing molecule. A careful study of the "free" spaces in membranes can be expected to disclose the types of monoreactive molecules that can enter membranes and reduce the harmful effects of the more toxic,reactive molecules.

AIRWAY HYPERIRRITABILITY INDUCED BY OZONE

by Lu-Yuan Lee, Eugene Bleecker, and Jay A. Nadel

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A characteristic feature of asthma is the extreme sensitivity of airways to stimuli such as histamine aerosols. We propose that damage of the airway epithelium is a cause of airway hyperirritability. Thus, we had shown that respiratory viral infections (which damage airway epithelium) cause transient airway hyperirritability in otherwise healthy subjects. Since ozone damages airway epithelium, we are studying its effect on airway irritability in dogs anesthetized with pentobarbital (25 mg/kg, iv), intubated, and ventilated with a Harvard respirator. Transpulmonary pressure was measured with an esophageal balloon, airflow with a pneumotachograph; total pulmonary resistance (R_L) was obtained by a method of electrical subtraction. Prior to ozone exposure inhalation of histamine diphosphate aerosol (5 breaths; 2% solution) increased R_L a mean of 335%. Sham exposure to filtered dry air did not change the bronchomotor response to inhaled histamine ($p > 0.5$). The dogs then breathed spontaneously from a free stream of ozone mixture (0.7–1.0 ppm) for two hours. In 8 experiments on four dogs, twenty-four hours after ozone, baseline R_L was not significantly changed ($p > 0.05$), but the response to histamine aerosol was greater than prior to ozone exposure (mean increase in R_L following histamine; 576%; $p < 0.01$). The increased bronchomotor response was maximum on the first day after ozone and returned to control levels in 7–28 days. Inhalation of isoproterenol aerosol (10 breaths; 0.5% solution) abolished the usual increase in R_L after histamine aerosol, suggesting that contraction of airway smooth muscle was responsible for the increase in R_L. We conclude that inhalation of relatively low concentrations of ozone causes transient airway hyperirritability in otherwise healthy dogs. These studies take on added importance in lieu of the fact that ozone has reached ambient levels as high as 0.54 ppm in Los Angeles and 0.4 ppm in other cities. Ozone may

play a role in the initiation of asthma and in the deterioration in the clinical state of asthmatic and bronchitic patients exposed to low levels of ozone in the ambient atmosphere.

Yi-Han Chang

CELLULAR ASPECTS OF INFLAMMATION

In the sequence of events that constitutes the inflammation process, the cellular response appears to be central and other events such as vasodilation, permeability augmentation, complement activation, mediator release, etc., facilitate the mobilization and localization of leukocytes at the inflammation site which involves a complex sequence of events including adherence to endothelium, diapedesis into the extravascular compartment, directional movement in response to chemotactic gradients, and phagocytosis of injurious material at the site of inflammation. The current understanding of inflammation is reviewed with emphasis on the cellular aspects. Experimental approaches and methodologies that proved useful in the elucidation of the mechanism of inflammatory response are discussed with a view toward possible application to the study of inflammation in the lung.

ABSTRACT

HUMAN BIOCHEMICAL EFFECTS OF GASEOUS POLLUTANTS

R. D. Buckley, J. D. Hackney, C. Posin, K. Clark

Healthy adult human subjects were exposed to low levels of selected air pollutants during a 2.5-3.0 hour regimen of exercise and pulmonary function tests. They breathed either filtered clean air (sham) only, or filtered air to which pollutants had been added. The temperature was maintained at 86°F and the relative humidity 43-47 percent. Several levels of pollutants were employed, although generally one at a time. The two most studied pollutants were ozone (O_3) and nitrogen dioxide (NO_2), whose concentrations did not exceed 0.5 ppm and 2 ppm respectively. Monitoring of pollutant levels was done continuously. Blood samples were taken at the end of each sham and exposure period and analyzed immediately. Paired group t-dependent statistical analyses were performed.

Statistically significant differences ($P < 0.05$) were detected in selected biochemical parameters of human blood following inhalation of both O_3 and NO_2. Two significant changes in the erythrocyte membrane (membrane fragility [RBC Frag.] and acetylcholinesterase [AcChase] activity) showed a dose-related response to O_3. Other changes did not show this relationship but behaved more like all-or-none responses. The data suggests that two basic kinds of responses to inhaled oxidant are seen in erythrocytes. The first is the initial injury or irritation and the second the response of the cell's biochemistry to that injury. Indirect evidence suggests that lipid peroxidation might be one of the primary causes of O_3 and NO_2 toxicity.

Additional studies compared the response of Los Angeles residents with subjects living in cleaner atmospheres and found that nonresidents showed responses that were similar in kind but greater in magnitude than those of local residents. This lower reactivity of Los Angeles residents suggests that adaptation to oxidant toxicity occurs upon prolonged breathing of the ambient local atmosphere, which contains appreciable O_3 and NO_2, as well as other irritant substances.

From the Specialized Center of Research in Environmental Lung Disease, National Heart and Lung Institute, Grant No. HL-15098-05.

Short-term effects of sulfuric acid aerosols. A morphological study in guinea pigs, mice, and rats.

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This study was designed to evaluate the morphological effects of sulfuric acid aerosols on the respiratory tracts of animals free of spontaneous respiratory disease and to establish an order of species susceptibility. Hartley albino guinea pigs, Sprague-Dawley rats, and Swiss-Webster mice were continuously exposed for periods ranging from 4 to 14 days to aerosols of H_2SO_4. Exposure details are included in Table I.

Table I. Exposure Summary

Animals	Aerosol Size (μm)	Mass Concentration (mg/m^3)	Exposure Length (days)
Rats	0.4 (~MMAD)[1]	68	6
Rats	0.45 (CMD)[2]	172	7
Rats	0.52 (CMD)[2]	45	11
Guinea Pigs	0.52 (CMD)[2]	71	4
Guinea Pigs	0.31±0.05 (MMAD)[1], Gg~1.6	30	7
Guinea Pigs	0.31±0.03 (MMAD)[1], Gg~1.5	38	7
Mice	0.62±0.04 (MMAD)[1], Gg~1.7	170	10
Mice	0.32±0.03 (MMAD)[1], Gg~1.4	140	14

[1]Mass Median Aerodynamic Diameter
[2]Count Median Diameter

Animals were confined within Hinners-type stainless steel exposure chambers, and acid aerosols were generated from vaporized SO_3 entering a humidified airstream.

Following exposure, animals were deeply anesthetized with pentobarbital sodium and euthanized by exsanguination. The lungs were excised as rapidly as possible, inspected for gross lesions, and perfused with Karnovsky's fixative via the airway at 30 cm of water pressure. For optimal perfusion, guinea pig lungs were submerged and perfused in fixative warmed to 37°C. Nasal septa were fixed by submersion. Blocks of tissue were selected for histiologic, scanning and transmission electron microscopic examination from nasal septa, trachea, and right middle and caudal lung lobes.

Results indicate a striking variability in species susceptibility to acid aerosol induced respiratory system damage. Guinea pigs were much more sensitive than the mouse or rat. Microscopic areas of erosion and

ulceration of bronchi and bronchioles, were frequently observed in large airways. Coagulation necrosis of bronchial epithelium, smooth muscle, and cartilage was present. Similar changes were observed in exposed mice, but the distribution differed in that lesions were confined to the larynx and adjacent trachea. The focal necrotizing laryngitis was associated with a prominent infiltration of edema fluid and neutrophils. The respiratory tracts of exposed rats were indistinguishable from those of controls.

Results suggest that large conducting airways of guinea pigs and the larynx of mice are sensitive regions of H_2SO_4 induced damage. The rat respiratory tract was remarkably resistant to effects of H_2SO_4 aerosols.

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In Vivo and In Vitro Effects of Sulfur Dioxide Upon Biochemical and Immunological Parameters

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A broad-spectrum, multi-disciplinary study of the effects of sulfur dioxide upon biological systems has been undertaken. Studies to date have encompassed various levels of biological organization. Results of these studies and the implications for human populations will be discussed.

The effects of inhaled sulfur dioxide upon alveolar macrophage functional capacity have been studied in the rat. These *in vivo* studies have been paralleled by *in vitro* exposures of baboon alveolar macrophages to sulfur dioxide. Parameters studied include cell physiological function by phagocytosis and respiration; biochemical studies of alveolar macrophage enzymes from various subcellular localizations (plasma membrane, cytoplasmic, lysosomal, secretory and microsomal) and RNA, DNA, and protein metabolism, immunological mechanisms (effects of *in vitro* SO_2 exposure upon macrophage migration and inhibition of migration in response to MIF); morphology, and dynamic physical measurements (spin labeling) of plasma membrane perturbation.

In addition, the effects of sulfur dioxide upon human lymphocyte membranes have been examined by the spin label technique and are consistent with an instantaneous denaturation of membrane protein resulting in rapid clearance from the membrane of the altered proteins.

Interactions of sulfur dioxide with biological systems and with atmospheric components have also been explored at the molecular level by a combination of spectroscopic techniques. Evidence for heretofore unreported reactions of sulfur dioxide with: 1) protein nitrogen, and 2) light of visible region wavelengths will be presented.

SULFUR DIOXIDE: A VIEW OF ITS REACTIONS WITH BIOMOLECULES

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Sulfur dioxide and related materials are present in the atmosphere of industrial centers. Together with other effluents, this complex of air pollutants can have adverse effects on health as many epidemiological studies indicate. In the attempt to understand the role and importance of various pollutants in producing such effects, exposure of test animals to single agents under controlled conditions has been frequently used as a model for study. The ambivalent results in the case of sulfur dioxide point out the problems inherent in this approach. It is argued that a knowledge of the potential biochemical effects of sulfur dioxide would greatly aid an examination of its possible influence on the health of organisms. Interestingly, the information about its biochemical reactivity comes largely from sources which have not been concerned with its environmental impact.

Sulfur dioxide is a highly reactive molecule. It reacts readily with water, aldehydes, ketones, disulfides, nicotinamide adenine dinucleotide, flavin adenine dinucleotide, and mononucleotides, vitamin K_3, thiamine pyrophosphate, cytosine, and epinephrine. In order to provide a perspective on the relative importance of these reactions, they are compared in terms of their thermodynamics and kinetics. Further depth is given to

the comparison by considering the plausible biotransformations of sulfur dioxide, a principal one being its conversion into cysteine-S-thiosulfate. It is possible that thiosulfate is generated from this species in the liver and that both S-thiosulfates and the thiosulfate anion serve as the direct precursors for the sulfite oxidase detoxification reaction which produces sulfate. Some chemical and biochemical properties of S-thiosulfates and thiosulfates are provided. Finally, these findings are organized in terms of the question of threshold of response of an organism to sulfur dioxide. The thermodynamic and kinetic properties of the various reactions described above are used in this analysis.

Biological Origin and Metabolism of SO_2

K. V. Rajagopalan

Because of the chemical reactivity of SO_2 (or bisulfite) it is of importance to determine whether intracellular HSO_3^- could attain concentrations at which it could be considered a threat to physiological constituents. Particularly, the finding that 1 M HSO_3^- can react with nucleic acid components raises the spectre of possible genetic hazards from atmospheric SO_2.

In animals HSO_3^- is generated endogenously from the amino acids methionine and cysteine. However, the presence of sulfite oxidase in animal tissues ensures that the HSO_3^- is rapidly oxidized to $SO_4^=$ and excreted. In studies on the effect of respired SO_2 and injected HSO_3^- on control and sulfite oxidase deficient rats it has been demonstrated that tissue sulfite oxidase is indeed capable of affording protection against the lethal effects of acute doses of these compounds. The extent of protection afforded by the enzyme suggests that the maximum *in vivo* activity of the enzyme approaches the value predicted from *in vitro* activity measurement.

The sulfite oxidase activity of adult human liver has the capacity for the oxidation of 4,000-8,000 mmoles of HSO_3^- to $SO_4^=$ per day. In comparison the average daily endogenous production of HSO_3^- in man is about 25 mmoles. Dietary consumption of HSO_3^- as food additive is only about 0.2 mmole per day. In human lung there is sufficient sulfite oxidase to convert 150 mmoles of HSO_3^- to $SO_4^=$ per day. In contrast, maximum HSO_3^- respired per day at 5 ppm SO_2 corresponds to only 1.3 mmoles. From a consideration of these data it may be concluded that significant accumulation of HSO_3^- in human tissues, at prevalent atmospheric concentrations of SO_2 or at the present levels of dietary intake of HSO_3^-, is extremely unlikely. These compounds therefore may not be classified as environmental genetic hazards. The possibility of damage to lung tissue from respired SO_2 needs to be evaluated.

CELLULAR MECHANISMS OF LUNG FIBROSIS

Milos Chvapil, M.D., Ph.D.

The process of lung fibrosis could be best controlled by affecting the early cellular stages of inflammation. Macrophages seem to play an active role in activation of fibroblasts - only activated macrophages stimulated collagen synthesis by fibroblasts. In the silica injured lung tissue, substances are formed at a certain time, which also specifically activate fibroblasts . This period coincides with macrophage accumulation in the lesion.

Among various trace elements, zinc administered *in vitro* to incubation medium or *in vivo* to intact animals exerts an inhibitory effect on various functions of cells, like mast cell, granulocytes, macrophages and platelets. A working hypothesis will be presented implicating trace elements in homeostasis of inflammatory cells. The importance of this mechanism to lung pathology will be discussed.

CELLULAR APPROACHES TO THE STUDY OF ENVIRONMENTAL POLLUTANTS. N.A. Elson and R.G. Crystal, NIH, Bethesda, Maryland 20014.

Exposure to environmental pollutants produce lung pathology with complex features including destructive lesions resembling emphysema, bronchiolar lesions, vascular lesions and proliferative lesions of the interstitium and epithelium. It is apparent that multiple cell types are involved and the changes seen undoubtedly represent a montage of the primary toxic effects of the noxious agent, secondary destructive effects of the inflammatory process, plus repair processes and fibrosis. The sorting out of these different processes is made more difficult by repeated exposure to the agent, resulting in superimposition of multiple stages of the process. However, to help evaluate the effects of environmental pollutants on the lung, several experimental approaches are now available to examine these complexities at the biochemical level.

One such approach is the analysis of the cellular and protein content of lung lavage. This technique is valuable because studies in the experimental model can readily be applied to human material through the use of fiberoptic segmental lavage. Preliminary human studies have shown that in idiopathic pulmonary fibrosis there is an increase in alveolar polymorphonuclear leukocytes, while in chronic hypersensitivity pneumonitis there is a striking appearance of lymphocytes, predominantly T-cells. Other studies have suggested that patients with pulmonary fibrosis have circulating T-lymphocytes which are sensitized to collagen. This raises the possibility of screening alveolar lymphocytes obtained for sensitization to specific extrinsic or intrinsic antigens which may be part of the disease process. Other studies indicate that alveolar macrophages and lavage fluid supernatant can be screened for specific enzyme activities (collagenase, neutral protease, elastase, β-glucuronidase, lysozyme) and for immunologic components (immunoglobulins, complement).

A second experimental approach is the short term lung explant. In this method, animal or human lung fragments are incubated in the presence of a specific radioactive precursor in order to quantitate rates and types of macromolecules being synthesized and degraded. Data to date indicates that important changes occur in rates of collagen and glycosaminoglycan synthesis with age in the developing animal, and that these rates can be altered in response to external stimuli e.g., post-pneumonectomy lung growth or radiation induced pulmonary fibrosis. These methods could easily be expanded to evaluate the effect of environmental pollutants.

A third approach is that of separating the explant model into its component cell constituents. Our laboratory has utilized several isolated cell types found in the lung, including the lung fibroblast, alveolar type II cell, alveolar macrophage, polymorphonuclear leukocyte and monocyte. Additional work is in progress on the isolation of a vascular endothelial and smooth muscle cell. These cells are maintained in short or long term culture, during which time they can be exposed to various environmental manipulations. The results of these manipulations are monitored by observing quantitative and qualitative alterations in the pattern of intracellular and secreted cellular proteins; this pattern serves as a "biochemical fingerprint" to detect the effects of injury at the cellular level.

BIOCHEMICAL MECHANISMS OF INTERACTION OF ENVIRONMENTAL METAL CONTAMINANTS WITH LUNG CONNECTIVE TISSUE

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Heavy metals are becoming increasingly significant as a potent hazard in the environment. The ions of these metals are involved in causing a wide variety of biological effects. As for example, Cd^{2+} decreases lucose metabolism, and affects respiration of pulmonary macrophages, uncouples oxidative phosphorylation and inhibits mitochondrial oxygen uptake. Cd^{2+}, Hg^{2+} and Pd^{2+} all interact with a large number of enzymes through -SH groups and destabilizes lysosomal membranes resulting in the release of lysosomal hydroxylases and impairing ion transport through membranes. These effects directly contribute to the biochemical injury in the lung which then evoke a repair response. Chronic exposure to the metal and continuous repair processes culminate in "fibrosis". We have examined this possibility in one of the steps in collagen synthesis. Hydralazine, shown by us to be a potent inhibitor of prolyl hydroxylase, a critical enzyme in collagen synthesis, elicits a fibrotic response in lungs, *in vivo*. We examined the effect of this drug on collagen synthesis in organ cultures of lung. Hydralazine stimulated the incorporation of labeled proline into the collagen sequence. The possibility that heavy metals may also act in the same manner was examined in terms of their interactions with purified prolyl hydroxylase *in vitro*. All these metals prove to be potent inhibitors of prolyl hydroxylase. The mechanisms involved a competition between the metal and Fe^{2+}, the prosthetic metal required for activating the enzyme. Our studies suggest that interference with prolyl hydroxylase may be an improtant part of biochemical injury leading to proliferation of connective tissues in lungs.
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Environmental Effects on the Biosynthesis of Lung Elastic Tissue

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The involvement of elastic tissue in the pathogenesis of various pulmonary diseases has recently gained the attention of many investigatiors. Lung elastic tissue has not been extensively studied or characterized since much of the work thus far has centered on the elastic tissue of aorta and ligament. For the last year our laboratory has been investigating the chemistry of the major protein component of elastic tissue, namely elastin. We have been particularly interested in comparing the chemistry and biosynthesis of lung elastin to elastins isolated from different tissues. Our initial experiments have centered on young chicks as a source of elastin since we have already accumulated much data on elastin synthesis, structure and degradation from these animals. To date we have been able to isolate soluble precursor forms of elastin from lung, aorta and gizzard tissues. Amino acid composition and molecular weights of the precursor molecules from each tissue are very similar if not identical. Antibody generated to aortic tropoelastin crossreacts and exhibits identity to the lung and gizzard samples. We have also been examining the effect of various toxic chemicals, such as lathrogens and metal chelators on the synthesis of lung elastin. Such studies should shed some light on the disease mechanism occuring in lung elastic tissue aberrations.

ABSTRACT FOR E.P.A. SYMPOSIUM

"Biochemical Effects of Environmental Pollutants: An E.P.A. Symposium"

ROLE OF NUTRITION IN HEAVY METAL TOXICITY

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The nutritional status of experimental animals must be considered of just as great importance in evaluating the toxicity of any agent, as is the health of the animals with respect to the presence of infection. Thus it has been shown that composition of the diet may modify the responses of animals to carcinogens and many heavy metals. A review of the literature shows that the dietary concentrations of protein, calcium, phosphorus, vitamins, and some essential transition metals will alter the toxic responses of several species of experimental animals to the oral ingestion of cadmium or lead. The toxicity of these metals when they are administered by inhalation has not been extensively studied.

In this presentation the roles of dietary concentrations of zinc, copper, and iron in affecting the toxicity of cadmium or lead will be emphasized. It will be shown that the toxicity of lead or cadmium is increased when the intake of one or more of these essential metals is reduced. It is known that females and children are more susceptible to lead toxicity than are males, and it is also known that these same portions of our population have at times a greater need for dietary zinc, copper, and iron; therefore, the possibility exists that better nutrition of the population as a whole with respect to essential transition metals may reduce the health hazards of cadmium and lead which are now dissipated in our environment and which may continue to be increased.

ALA-D IN LEAD EXPOSURE

By

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Lead at very low concentrations is known to inhibit δ-Aminolevulinate dehydratase (ALA-D) which catalyses the formation of porphobilinogen, a precursor of heme. At least one more enzyme involved in the pathway of heme synthesis is inhibited by lead, that is ferrochelatase which catalyses the insertion of iron in the protoporphyrin IX to form heme. Due to the lead inhibition of these enzymes, the elevation of heme precursors in blood and urine can be detected in lead exposed men and animals.

Among the many indices utilized for the assessment of lead absorption such as blood lead levels, urinary lead, urinary ALA, zinc protophorphyrins (ZPP) etc., erythrocyte ALA-D activity is the method of choice for low levels of exposure since its inhibition by lead is highly specific and sensitive.

Numerous investigators have reported that a slight increase in lead absorption is associated with a significant depression of ALA-D activity. However, some other factors may affect the activity of this enzyme: Acute alcoholism, tyrosinemia and zinc deficiency could significantly decrease ALA-D.

This is a sulfhydryl containing enzyme and recently we have found it to be zinc dependent. In rats under controlled nutritional intake of zinc ALA-D is positively correlated with dietary zinc levels. Furthermore, <u>in vitro</u> addition of $ZnCl_2$ to hemolysates of blood from lead exposed animal brings ALA-D activity back to control values.

A study on one occupationally lead-exposed worker confirmed the above findings. Two important questions must be raised in consideration of these findings: a) should chelation therapy, which drastically increases urinary zinc excretion, be accompanied with administration of zinc and b) should the nutritional state of the exposed population, at least in regard to zinc, be considered in order to determine the biological importance of exposure levels. Current studies in our laboratories are aimed at answering these questions.

BIOINORGANIC CHEMICAL REACTIONS IN THE ENVIRONMENT

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The chemical behavior of any element in the environment depends on its form. For natural elements biogeochemical cycles have evolved which lead to steady state concentrations of individual species in metabolism. For the heavy metals and metalloids coordination complexes and organometallic complexes are of prime importance, especially the latter because metal-alkyls have a tendency to bioaccumulate as a result of their solubility in lipids.

During the last ten years my research group has delineated metabolic pathways leading to the biosynthesis of methyl-mercury, methyl-arsenic, methyl-selenium and methyl-tin compounds. Recently, we have turned our attention to enzyme systems which dealkylate metal alkyls. From our study it is clear that the kinetics for the biosynthesis of metal-alkyls in the environment, and the kinetics for the uptake of metal-alkyls in specific ecosystems represent fundamental problems which must be faced. Monitoring steady state concentrations of elements which enter natural biological cycles yields information which is of limited value, since the kinetics of uptake by living systems will always determine environmental impact.

In this lecture I shall deal with the kinetics and mechanisms for the biosynthesis of metal-alkyls. In addition I shall discuss recent work on enzyme systems which dealkylate metal-alkyls, followed by reduction of inorganic salts of heavy metals to their lowest oxidation states. Preliminary work on the possible alkylation of lead in well-defined biochemical systems will be discussed.

Effects of Heavy Metals on Isolated Mitochondria

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Under appropriate experimental conditions, a number of heavy metals, including Pb, Cu, Hg, Cd, Ag and Zn, have been shown to inhibit mitochondrial enzyme and transporter activities and to alter the permeability of the inner membrane to cations and anions. In several cases, there is reason to infer that such reactions are directly related to the toxicity of the metal *in vivo*. The observed effect *in vitro* of a given concentration of a metal ion, such as Pb, will reflect an interplay between the following factors: (a) The composition of the suspending medium - In the presence of Pi, for example, Pb^{+2} is effectively removed from solution and has little effect on mitochondria whereas Zn^{+2} becomes more reactive when adsorbed on the membrane as a phosphate complex. (b) The extend of reaction of the metal with membrane binding sites - There are over 100 potential binding sites per mg of protein for a divalent cation in isolated heart mitochondria. Reaction of 5-10 of these sites with Cu^{+2}, for instance, produces an increased passive permeability to K^{+} and markedly stimulates the energy-dependent uptake of this cation. As more Cu^{+2} is reacted with the membrane, permeability to anions and loss of energy-coupling are noted. The energized and non-energized membrane often show a different reactivity to a given amount of heavy metal, suggesting that different ligands are available to the metal under these two conditions. (c) The ability of the metal ion to penetrate into the matrix compartment - Zn^{+2} and Pb^{+2} have been shown to be accumulated by an energy-dependent process and an extensively reacted membrane will probably permit passive inward diffusion of the metal ions. (d) The intrinsic susceptibility of primary dehydrogenases and other matrix enzymes to inhibition by the metal ion in question.

ABSTRACT

Metabolic Interactions of Selenium with Heavy Metals

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Mortality and other toxic symptoms of cadmium, mercury, silver and thallium in animals are much reduced by administration of selenium. Even though selenium protects against heavy metal toxicity, higher concentrations of these metals accumulate in tissues of selenium treated animals than in untreated ones. Studies on the mechanism(s) for the protective role of selenium in heavy metal toxicity have shown that selenium alters tissue and subcellular distributions of cadmium and mercury and changes the binding of these metals to constituents of the cell cytosol. The physiological significance of some of these results is questionable, however, since in many cases the animals were injected with selenium and the heavy metal. For example, redistribution of cadmium or mercury from low to high molecular weight proteins in the cytosol occurs when selenium and the metals are injected but not when these elements are given orally.

The mechanism for the protective effect of selenium apparently differs, depending on the chemical form of the metal. Treatment of animals with inorganic mercury or cadmium and selenium causes both tissue and subcellular redistribution of the metals whereas administration of methylmercury and selenium alters only the tissue distribution of methylmercury. Selenium shifts much of a dose of methylmercury into muscle but causes inorganic

mercury to concentrate primarily in liver and kidney. The relative effectiveness of selenium compounds in modifying the distribution of inorganic mercury in tissues is in the decreasing order: selenomethioneine > selenocystine > selenate > selenite.

Cadmium, silver and mercury also reduce the toxicity and the availability of selenium as well as changing the tissue distribution of this element. In other work, silver and mercury markedly decreased the activity of glutathione peroxidase, a selenoenzyme, whereas cadmium had no effect.

It thus appears that selenium does not counteract heavy metal toxicity by a single, common mechanism. Furthermore, the metal and its chemical form, the route and time sequence of administration and the relative dosages of selenium and metal all influence the observed response.

Platelet and RBC Metabolism

In The Study of Molybdenum and Other Trace Substances

Clive C. Solomons, Ph.D.

Blood cells from rats and humans were used as models for measuring the effects of molybdenum. Changes in platelet purine metabolism of industrial workers and students correlated well with their plasma uric acid concentrations (r=0.77). Less significant correlations of uric acid and xanthine oxidase activities of red blood cells were also seen (r=0.44). Human blood cells were found to differ significantly from those of rats in the Mo-dependent uric acid pathway and the stress of obtaining blood from the rats could also alter the measurement of platelet function and metabolism in an uncontrolled fashion.

It was concluded (i) that enzymatically-controlled reactions of human platelets appeared to be meaningfully correlated with Mo metabolism and uric acid levels. This system appears to be capable of further development for detecting exposures which could result in blood Mo levels in the 5-50 ppb range. (ii) that the rat is not a suitable model for evaluating Mo effects on human populations, although the opportunity to study rats was a key factor in optimizing the analytical methods. (iii) that further work should proceed relating Mo effects in the 0-100 ppb range on human platelets in order to define the basic mechanism of Mo in ATP metabolism and develop a simple field test for detecting exposure to Mo. The effects due to Mo deficiency and Mo excess could be evaluated in human populations living in different geographical areas or receiving different occupational exposures to Mo.

Neurotransmitter Mechanisms in Inorganic Lead Poisoning

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Mice, rats and monkeys chronically exposed to lead during critical periods of development present with increased levels of spontaneous motor activity (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973; Allen, McWey and Suomi, 1974). In the mouse, lead induced hyperactivity is suppressed by drugs that enhance central cholinergic function (e.g. physostigmine) and is exacerbated by anticholinergic drugs (e.g. benztropine) and aminergic agonists (e.g. l-dopa) (Silbergeld and Goldberg, 1975). This experimental hyperactivity, however, presents a paradoxical pharmacology in that it is suppressed by methylphenidate and amphetamine, drugs classically thought of as aminergic agonists (Silbergeld and Goldberg, 1974).

Animals chronically exposed to lead appear to have enhanced aminergic function. The turnover (Golter and Michaelson, 1975) and steady-state levels of norepinephrine (Golter and Michaelson, 1975; Silbergeld and Goldberg, 1974) are increased. In addition, the excretion of vanillylmandelic acid and homovanillic acid, metabolites of norepinephrine and dopamine, are increased in lead exposed mice and children (Silbergeld and Chisolm, 1976). Lead has also been shown to impair cholinergic function by blocking acetylcholine release in the superior cervical ganglion (Kostial and Vouk, 1957), nerve-muscle preparations (Mannelis and Cooper, 1974; Silbergeld, Fales and Goldberg, 1974) and in brain preparations (Carroll, Silbergeld and Goldberg, 1976).

This paper will summarize the data dealing with neurotransmitter function in lead induced hyperactivity. The emphasis will be on cholinergic aspects and the interaction between cholinergic and aminergic function. Supported in part by grants from the National Institute of Health EHS00034 and 00454.

EVALUATION OF ANIMAL MODELS USED TO STUDY EFFECTS ON NEUROCHEMISTRY AND BEHAVIOR

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ABSTRACT

Inorganic lead produces cerebral dysfunction and clinically definable encephalopathy. It is not known, whether chronic low level lead exposure has an adverse effect on brain maturation of children. Studies attempting to define behavioral and/or neurochemical changes following lead exposure have been primarily conducted in the neonatal rat or mouse.

Newborns are frequently exposed to lead *via* milk from dams consuming lead in food or drinking water. Sucklings are often weaned to and maintained on the dam's lead source. Young adult rats and mice exposed in this manner have been reported to be hyperactive, exhibit disruptions in normal brain neurotransmitter metabolism and display "paradoxical" responses to stimulants and depressants. These lead exposed animals show growth retardation.

Control subjects are matched for age but seldom are they matched for growth rate. Undernutrition, wherein growth retardation is comparable to that seen in lead

exposed mice can lead to enhanced spontaneous locomotor activity, apparent "paradoxical" response to d-amphetamine and enhanced activity following phenobarbital. These data suggest that some of the behavioral and pharmacological responses seen in lead exposed rodents can be explained on the basis of early undernutrition.

EFFECTS OF TRACE METALS AND THEIR DERIVATIVES ON THE CONTROL OF BRAIN ENERGY METABOLISM

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A means has been devised whereby changes induced in the redox state of the mitochondrial electron transport intermediates can be measured within slices of brain tissue (Bull and Lutkenhoff, J. Neurochem. 21,913-22 1973) Spectral changes induced by elevated media potassium concentrations (3 to 30 mM) parallel closely changes induced in more conventional measures of metabolism such as oxygen consumption, aerobic glycolytic rate, adenine nucleotides and phosphocreatine. This provides a technique which can be used to detect subtle changes in the relationship of function to metabolism in small samples of brain tissue taken from animals exposed to environmental chemicals.

$PbCl_2$, administered I.P to Charles River CD strain rats over a two week period to brain levels of lead approximating 0.41 ug/g fresh weight or higher, results in alterations in the metabolic responses of cerebral cortical slices to potassium. The effect is observed as an inhibition of NAD(P)H oxidation which is accompanied by an inhibition of potassium-induced respiration. This inhibition is paralleled by a decrease in the rate of potassium-induced calcium turnover in the tissues that is closely associated in time with the respiratory burst produced by elevated potassium. Previous work has implicated a calcium component in the respiratory response to potassium (Bull and Cummins, J. Neurochem. 21,923-37 1973).

Methyl mercury administered daily for a two week period in doses greater than 0.05 mg/Kg per day gave rise to cortical Hg concentrations of 0.1 ug/g fresh weight. At these levels, changes in the spectral responses of cortical slices were seen to be altered in a manner similar to that observed with lead. The accumulation of NAD(P)H produced by low doses of methyl mercury gradually decreased towards control levels with higher doses. This latter effect was associated with a decrease in the tissues' capabilities for aerobic glycolysis.

Dialkyltin compounds, used as stabilizers in PVC pipe, have been compared, in vitro, with triethyltin, a known C.N.S. toxin. Triethyltin half-maximally inhibits NAD(P) reduction induced by potassium at a concentration of 1.1 uM (0.12 mg Sn/liter). This effect is opposite to what is observed with lead and methyl mercury either in vivo or in vitro. Dimethyltin and dibutyltin were observed to produce the same effect as triethyltin at concentrations of 66 and 45 uM, respectively (7.9 and 5.3 mg Sn/liter).

The present results suggest that the trace metals studied interfere with the control of energy metabolism within brain tissue. These effects may be direct or indirect, but it is clear that coupling of function to metabolism is affected at doses well below those which give rise to overt toxicity.